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<b>(21) International Application Number:</b> PCT/EP99/00782 <b>(22) International Filing Date:</b> 5 February 1999 (05.02.99) <b>(30) Priority Data:</b> MI98A000234 6 February 1998 (06.02.98) IT <b>(71) Applicant (for all designated States except US):</b> VECTOR-PHARMA S.P.A. [IT/IT]; Via del Follatoio, 12, I-34148 Trieste (IT). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> ESPOSITO, Pierandrea [IT/IT]; Via Montona, 4, I-34100 Trieste (IT). COLOMBO, Italo [IT/IT]; Via Don Sturzo, 15, I-20065 Inzago (IT). COCEANI, Nicoletta [IT/IT]; Via Firmano, 10, I-33043 Cividale (IT). DEL CURTO, Maria, Dorly [IT/IT]; Via Oratorio, 4, I-27050 Corvino Sanquiro (IT). CARLI, Fabio [IT/IT]; Salita Cedassammare, 3/1, I-34136 Trieste (IT). <b>(74) Agent:</b> GERVASI, Gemma; Notarbartolo & Gervasi S.p.A., Corso di Porta Vittoria, 9, I-20122 Milan (IT).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> PHARMACEUTICAL COMPOSITIONS IN FORM OF NANOPARTICLES COMPRISING LIPIDIC SUBSTANCES AND AMPHIPHILIC SUBSTANCES AND RELATED PREPARATION PROCESS		
<b>(57) Abstract</b>  Pharmaceutical compositions in form of nanoparticles comprising a composite material, consisting of at least one lipidic substance and of at least one amphiphilic substance, and of a pharmaceutically active principle. Said compositions, thanks to the surface and mass properties of said composite material, show an improvement in the incorporation of the active principles and an increase in the bioavailability of the poorly absorbable active principles.		

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PHARMACEUTICAL COMPOSITIONS IN FORM OF NANOPARTICLES  
COMPRISING LIPIDIC SUBSTANCES AND AMPHIPHILIC SUBSTANCES AND  
RELATED PREPARATION PROCESS

**Prior art**

5 In the research field of the new vehicles suitable to the administration of active principles, a great interest has been directed towards the polymeric systems having size in the micrometer range and to the polymeric systems having size in the nanometer range.

Among the mostly used polymers the polyalkylcyanoacrylates and the poly-lactic  
10 acid (PLA) and poly-lactic glycolic acid (PLA-PLGA) derivatives are to remember. Such systems show however some disadvantages.

For example the polyalkylcyanoacrylates are metabolized by the organism in a 24 hours interval and release formaldehyde, a potentially toxic derivative; the PLA and PLA-PLGA polymers do not produce toxic metabolites but they have long  
15 degradation times ranging from some weeks to some months, and then they may show dangerous accumulation phenomena.

Moreover, the preparation methods of these systems need the use of potentially toxic organic solvents which may remain in traces in the final form.

In the end, the size of the majority of said systems exclude their use for  
20 intravenous way because extraneous bodies having size higher than 5  $\mu\text{m}$  injected in vein may cause embolisms.

These negative aspects generated greater attention for administration systems having greater biocompatibility and lower toxicity: the first among all these are the lipidic colloidal systems such as oil/water emulsions, liposomes, lipidic micro- and  
25 nanoparticles.

Oil/water emulsions, consisting of lipidic droplets having size in the range of nanometers, dispersed in an external aqueous phase, have been used as a vehicle for the parenteral feeding (JP Patent No. 55,476, 1979, Okamota, Tsuda and Yokoama).

30 Oil/water emulsions containing active principles have been described in the Patent WO 91/02517, 1991, Davis and Washington. Such systems have a high capacity

to incorporate active principles in the internal lipidic phase, but the active principles easily diffuse from such phase towards the external phase originating stability problems and limitations for the optional development of a protracted release form.

5 The liposomes are colloidal structures having an aqueous internal phase surrounded by one or more layers of phospholipids. The use of liposomes as vehicles for the administration of drugs is described for example in the U.S. Patent No. 3,993,754 (1976, Rahman and Cerny).

However typically, such systems show stability problems during the stocking, a  
10 poorly reproducible preparation method and a low potentiality to incorporate and retain active principles.

Fountain and others invented lipidic microparticles in globular form having size ranging from 0.5  $\mu\text{m}$  to 100  $\mu\text{m}$  as vehicles for the administration of active principles. Such invention is disclosed in the U.S. Patent No. 4,610,868 (1986).

15 Domb and others (US Patent 435,546) invented the Liposheres™, insoluble particles having size about equal to 40  $\mu\text{m}$ , suspended in an aqueous environment, consisting of a lipophilic internal phase surrounded by external layers of phospholipids, added to the composition and adsorbed on the surface of the particles themselves. These systems were developed for the controlled  
20 release of anaesthetic drugs (Domb and others, US Patent 5227165) and of active principles having insecticide and pesticide activity (Domb and others US Patent 5227535). However the technique for the preparation of such systems requires the help of solvents which remain in traces in the final form.

The per os administration turns out to be difficult for active principles which are not  
25 much soluble, not much absorbed in the gastroenteric tract or which are sensible to the pH or the action of the proteolytic enzymes (proteins and peptides). The incorporation of such substances in lipidic nanoparticles allows to overcome such difficulties because these nanoparticle systems may be absorbed along the gastrointestinal tract. Their reduced size allow to exploit the mechanisms of the  
30 passive transmucosal absorption, or to pass through the intercellular junctions or the ionic channels or to use the endocytosis mechanism or to enter the lymphatic

flux.

Solid lipidic systems consisting of nanopellets were developed by Speiser and others (US Patent 4,880,634, 1989), and destined to the oral administration of poorly absorbed drugs. The lipidic pellets are prepared emulsifying lipidic substances in an aqueous environment with a high energy mixer, then cooling the emulsion at room temperature and obtaining the pellets by sonication.

Gasco (EP 0526666A1, 05/08/1991) invented a technique for the preparation of lipidic nanoparticles. A microemulsion is prepared adding to an aqueous phase a lipid melted in the presence of surfactants and cosurfactants, which is then dispersed in an aqueous environment maintained at a temperature around 10 °C. The solid nanoparticles are obtained in an aqueous suspension, but may be subsequently deprived of the residual surfactants by ultrafiltration and recovered by filtration or freeze-drying.

Such technique turns out to be advantageous from the point of view of the saving of energy with respect to the high energy homogenization, it allows to obtain smaller nanoparticles, having average diameters ranging from 90 nm to 900 nm, with a more uniform size distribution and a low polydispersion index. However the preparation of a microemulsion needs the melting of the lipidic material which is for most used lipidic substances about 70 °C, which limits the use of such technique for the thermolabile substances.

### **Summary of the invention**

The invention relates to pharmaceutical compositions in form of nanoparticles, having a diameter lower than 1000 nm and preferably ranging from 50 to 500 nm, comprising a composite material, consisting of at least one lipidic substance and at least one amphiphilic substance, and a pharmaceutically active principle.

We have unexpectedly found that, operating according to the present invention, said composite material and the relative particles have characteristics not achievable by an usual mixing of a lipidic substance with an amphiphilic substance or by the adsorption of an amphiphilic substance on lipidic particles.

The amphiphilic substance may be preferentially distributed on the surface of the nanoparticles or it may be preferentially distributed inside the nanoparticles or it

may be homogeneously distributed on the surface of and inside the nanoparticles. The formation of the composite material allows to obtain nanoparticles:

1. with surface characteristics helping the oral administration absorption and the half-life time in the circulatory system;
- 5 2. with mass characteristics, as the low melting temperature, allowing to incorporate thermolabile drugs;
3. suitable, thanks to the presence of lipophilic zones and partially hydrophilic zones in the composite material, to the vehiculation both of hydrosoluble drugs and of liposoluble drugs;
- 10 4. able to homogeneously incorporate the hydrophilic drugs (for example peptides) inside an essentially lipophilic matrix.

#### **Detailed description of the invention**

The invention relates to the preparation of compositions for pharmaceutical use in form of particles having size lower than one micrometer (nanoparticles),  
15 comprising a composite material consisting of lipidic and amphiphilic substances, the latter being of lipidic or polymeric kind.

Generally, the nanoparticles according to the invention are prepared starting from a composite material obtained by comelting or cosolubilization of the lipidic material and the amphiphilic substances. The comelted mixture, at the subsequent  
20 cooling, results in a composite material having new characteristics with respect to the two starting materials, showing more hydrophilic zones and more lipophilic zones thanks to the reciprocal disposition of the components or to the segregation of the amphiphilic material towards the surface or inwards the mass of the nanoparticles. These characteristics are substantially different from the surface  
25 adsorption of an amphiphilic substance on a lipophilic surface. Such properties will be described in detail in the Characterization Examples reported below.

The drug may be dissolved or suspended in said comelted mixture during the preparation process and, thanks to the new properties of the composite material, it may divide, according to its characteristics, preferentially inside the more  
30 hydrophilic areas or the more lipophilic areas. Moreover, the hydrophilic drugs (for example peptides) charged on the nanoparticles turn out to be unexpectedly

distributed in an homogeneous way inside the nanoparticles themselves while the peptide fraction adsorbed on the surface turns out to be very low, and lower with respect to the Examples of the prior art (particles consisting of lipidic core and adsorbed amphiphilic substance).

5 The nanoparticles obtained from the comelted mixture maintain the same characteristics of the starting composite material.

The nanoparticles may be obtained with different preparation techniques:

- 1. a technique providing for the dispersion of a oil in water microemulsion (consisting of, as oil phase, lipidic and amphiphilic materials kept at a temperature  
10 higher than the melting point of the composite mixture and one or more surfactants and cosurfactants) in an aqueous medium, utilising the temperature gradient.

- 2. A technique providing for the high pressure homogenization of a fine emulsion of a composite material, at a temperature higher than the melting temperature of  
15 the materials forming the composite, or of a fine suspension of a composite material, below the melting temperature of the composite, in presence of surfactant agents.

The preparation of the invention according to the microemulsion-dispersion process (technique 1), provides for the initial comelting or cosolubilization of two  
20 or more lipidic and amphiphilic components, taken to the melting temperature of the components themselves or at least to the melting of one of the two components, when the latter is soluble in the former; an appropriate volume of an aqueous solution containing one or more surfactants and cosurfactants, warmed at the same temperature of the composite material melted, under mild stirring is  
25 added to such melted composite material.

It is also possible to form the microemulsion simultaneously taking to the melting temperature the lipidic and amphiphilic components in presence of the water and the surfactants and cosurfactants needed to formation of the microemulsion itself.

The active pharmaceutical principle may be dissolved or dispersed in the starting  
30 melted composite material or added directly to the microemulsion during the preparation of the microemulsion itself, depending on the properties of the active

principle itself. The distribution of the active principle occurs into the composite material, allowing an unexpected decrease of the drug amount adsorbed on the surface and submitted to the degrading action of the enzymes and of the external environment.

- 5 The so formed oil/water microemulsion is subsequently dispersed in water or in aqueous medium, in controlled volume and stirring conditions, at a temperature generally ranging from +1° to +10 °C, but that may also range from -15 to -30 °C using non aqueous solvents miscible with water, originating in this way the composite nanoparticles in solid form in aqueous suspension. Said nanoparticles
- 10 have a diameter lower than 1000 nm. The nanoparticles turn out to be different with respect to the systems obtained by the techniques of amphiphilic substances adsorption on the surface of the lipidic particles (Domb) or by the use of amphiphilic substances as surfactants for the formation of lipidic nanoparticles (Gasco).
- 15 Subsequently, the nanoparticle suspensions may be washed with water or aqueous solutions through an ultrafiltration system (or dialysis) which allows to remove the surfactant, cosurfactant and free drug excess. Therefore such process allows to remove the undesired possible effects due to the surfactants presence in the pharmaceutical form. Moreover, with such a procedure it is possible to
- 20 quantitatively determine the percentage of the active principle not incorporated or adsorbed on the nanoparticles.

The composition described above may be administered as an aqueous suspension or it is recovered as a solid by freeze-drying, filtration, evaporation of the aqueous solvent or spray-drying techniques.

- 25 The nanoparticles according to the present invention have the following quantitative composition by weight:
- lipidic substances from 0.5 to 99.5%, and preferably from 10% to 90%;
  - amphiphilic substances from 0.5 to 99.5% and preferably from 10% to 90%;
  - pharmacologically active principle from 0.001 to 99%, and preferably from 0.01%
- 30 to 50% with respect to the sum of the lipidic substances and amphiphilic substances.



In the preparation process of the nanoparticles according to the technique (1), the component substances are used in the following proportions by weight:

in the microemulsion:

- lipidic components, from 0.1% to 50% by weight and preferably from 10 to 25%;
- 5 - amphiphilic components, from 0.1% to 50% by weight and preferably from 0.5% to 25%;
- surfactants, from 5% to 30% and preferably from 10% to 20%;
- cosurfactants, from 0% to 15% and preferably from 3% to 7%;
- water, or aqueous solutions, from 40% to 75% by weight and preferably from
- 10 50% to 70%;
- pharmaceutical active principles, directly incorporated in the composite material or dissolved in the microemulsion, in concentrations variable on the base of the incorporation efficacy and of the desired dosages and ranging from 0.001% to 99% and preferably from 0.001% to 50% by weight with respect to the sum of the
- 15 lipidic components and amphiphilic components.

In the dispersion:

- the microemulsion prepared as described above is dispersed in aqueous environment (water or aqueous solutions) with volumetric dilutions from 1:2 to 1:200, preferably from 1:5 to 1:50.
- 20 To the dispersion
- coadjuvants of the dispersion, from 0.05% to 5% by weight;
- viscosizing agents, of polymeric kind, from 0.05% to 5% by weight may be added.

The preparation according to the high pressure homogenization technique

25 (technique 2) provides for the dispersion of the composite material, added with one or more adjuvant substances, in an aqueous environment. The composite material is homogenized to form nanoparticles maintaining the system at the melting temperature of the material itself or just below such temperature ("softening") or at temperatures maintaining the composite material at the solid

30 state.

The composite material may be initially prepared by comelting or cosolubilization,

analogously to what is reported for the technique 1, proceeding to the comelting of two or more lipidic and amphiphilic components taken to the melting temperature of the components themselves or at least to the melting of one of the two components when the latter is soluble in the former.

5 The composite material may be preliminarily dispersed in an aqueous solution containing surfactant substances, stabilizing substances and/or viscosizing substances by dispersion or low energy homogenization techniques (for example using Silverson L2R or Ultra-Turrax kind equipments). After such treatment, which may be not necessary if the composite material shows surface characteristics  
10 such as to help its dispersion in water, the system is submitted to high pressure homogenizator (for example of APV Gaulin, APV Rannie Mini-Lab, Microfluidizer kind) to repeated homogenization cycles which cause nanoparticle dispersions. The high pressure homogenization treatment may occur at the composite material melting temperature, at "softening" temperature or at temperatures at which the  
15 material is present in a solid state in micronized form.

The active principle may be comelted, dissolved or dispersed in the composite material or in each of its constituents during the comelting of the system, or added during the subsequent process phases, as it is or in presence of surfactants which helps its incorporation in the nanoparticles or the adsorption on their surface.

20 Subsequently, the nanoparticle suspensions may be washed with water or aqueous solutions, analogously to what is described for the technique (1), through a ultrafiltration system.

Analogously, the composition may be administered as aqueous suspension or recovered as a solid by freeze-drying, filtration or aqueous solvent evaporation or  
25 spray-drying techniques.

In the preparation process of the nanospheres according to the technique (2), the substances composing the invention are used in the following proportions by weight:

- lipidic components, from 0.1% to 50% by weight, preferably from 0.5 to 15%;
- 30 - amphiphilic components, from 0.1% to 50%, preferably from 0.5% to 15%;
- surfactants, from 0.05% to 10%, preferably from 0.5% to 5%;

- water, or aqueous solutions of hydrosoluble components, from 45% to 99.5%, preferably from 50% to 80%;
- dispersion coadjuvants from 0.05% to 5%;
- viscosizing agents, of polymeric kind from 0.05% to 1%;
- 5 - pharmaceutical active principles, directly incorporated in the composite material or dissolved in the microemulsion, in concentrations variable on the base of the incorporation efficacy and of the desired dosages, and ranging from 0.001% to 99.9% and preferably from 0.001% to 50% by weight with respect to the sum of the lipidic components and the amphiphilic components.
- 10 Among the lipidic materials usable according to the invention we can mention both natural products and synthetic or semi-synthetic kind products definable as "fats" in that they are not miscible or only partially miscible with water:
  - 1) natural fats either saturated or unsaturated and partially or totally hydrogenated vegetal oils, for example hydrogenated cotton oil (Lubritab™), hydrogenated palm oil (Dynasan™ P60) and hydrogenated soy-bean oil (Sterotex™ HM);
  - 15 2) semi-synthetic and synthetic mono-, di- and triglycerides containing saturated and/or unsaturated fatty acids (having aliphatic chain length ranging from C<sub>10</sub> to C<sub>22</sub>) and their polyhydroxyethylated derivatives, for example tristearine, caprico-caprylic triglycerides (Mygliol™, Captex™, Labrafac™ Lipo), behenic triglycerides (Compritol™) monoglycerides as glyceril monostearate (Myvaplex™ 600) or glyceril palmitostearate (Precirol™) and saturated or unsaturated polyhydroxylated triglycerides (series of Labrafil™, Labrafac™ Hydro, Gelucire™);
  - 20 3) "liquid waxes", for example isopropyl myristate, isopropyl-caprylate, -laurate, -palmitate, -stearate and esters of fatty acids, such as ethyl oleate and oleyl oleate;
  - 25 4) "solid waxes", for example carnauba wax and bees-wax;
  - aliphatic alcohols, for example cetyl alcohol, stearyl alcohol, lauryl alcohol, cetylstearyl alcohol and their polyhydroxyethylated derivatives;
  - 6) aliphatic carboxylic acids preferably having medium and long chain (C<sub>10</sub>-C<sub>22</sub>),
  - 30 saturated (decanoic acid, lauric acid, palmitic, stearic, docosanoic acid, etc.), unsaturated (oleic, linoleic, etc.) and their polyhydroxyethylated derivatives.

Among the amphiphilic materials of lipidic kind one can use lipids having in their structure some hydrophilic components, such as for example:

1) phospholipids belonging to the series: phosphatidyl glycerol, phosphatidylcholine and phosphatidic acid (e.g. dimiristoyl phosphatidyl glycerol);

5 2) mono- and di-glycerides such as glyceril monostearate (Myvaplex™ 600) or glyceril palmitostearate (Precirol™);

3) triglycerides and saturated or unsaturated polyhydroxylated triglycerides (e.g. series of Labrafil™, Labrafac™ Hydro, Gelucire™);

10 4) esters of fatty acids, such as decylester of oleic acid: Cetiol™ V and isopropylmyristate;

5) medium chain fatty acids (such as capric, caproic and lauric acids).

Among the amphiphilic materials of polymeric kind, polymers may be used such as:

15 1) Polyethylene glycols (PEG), both liquid (from PEG 200 to PEG 1000) and solid (from PEG 1500 to PEG 20,000);

2) poly-(propyleneoxide) poly-(ethyleneoxide) copolymers, Poloxamer (Lutrol™ 188, Lutrol™ 407);

3) Polyvinyl alcohol;

4) Polyacrylates (Carbopol™, Pemulen™, Noveon™);

20 5) Poly-(methylvinyl ether) -maleic anhydride (Gantrez™) copolymers;

6) Polysaccharides of natural origin such as chitosan and derivatives, ialuronic acid and derivatives, xanthan, scleroglucan, gellan, guar gum, locust bean gum, alginate and dextran;

7) Polyesters such as for example poly-ε-caprolactone.

25 As reported above the composite materials according to the invention may be prepared by mixing, comelting or cosolubilization of the components selected among the lipidic materials and among the amphiphilic materials of lipidic or polymeric kind. For example, composite materials according to the invention, may be formed from mixtures of fatty acids (stearic acid-decanoic acid), of fatty acids  
30 and phospholipids (stearic acid-dimiristoyl phosphatidyl glycerol or dimiristoyl phosphatidylcholine), fatty acids and triglycerides or polyhydroxylated triglycerides

(stearic acid and Labrafil™ 2130); mono- or di-glycerides with fatty acids (glyceril palmitostearate with stearic acid), fatty acids with polyethylene glycol (stearic acid-PEG), mono and diglycerides with copolymers of polyethylene oxide-polypropylene oxide (glycerilpalmitostearate with poloxamer).

5 As aqueous phases according to the invention (aqueous phase of the microemulsion according to the technique 1 and/or dispersing phase according to the techniques 1 and 2) may be mentioned:

- 1) water as it is or buffered at different pH and ionic strength;
- 2) aqueous solutions of hydrophilic, hydrosoluble or hydrodispersable polymers  
10 such as polyethylene glycol, polyvinyl pyrrolidone, polyacrylic acids and derivatives (e.g. Carbopol®, Pemulen®, etc.), polymethacrylic acids and derivatives (e.g. Eudragit®), copolymers of polyoxyethylene-polyoxypropylene (e.g. Poloxamer, Lutrol®), polysaccharides of various nature such as for example dextran, xanthan, scleroglucan, gum arabic, guar gum, chitosan, cellulose and  
15 starch derivatives;
- 3) aqueous solutions of saccharides (e.g. sorbitol, mannitol, xylitol);
- 3) mono or polyhydroxylic aliphatic alcohols, preferably having short chain (C<sub>2</sub>-C<sub>4</sub>);
- 4) polyethylene glycols (e.g. PEG 200, PEG 400, PEG 600, PEG 1000);
- 20 5) polyglycolic glycerides (e.g. Labrasol™);
- 6) polyglycols, such as for example propylene glycol, tetraglycol, ethoxydiglycol (Transcutol™).

Among the surfactants, to use in techniques 1 and 2, we may not exhaustively mention all the non ionic surfactants with a HLB value generally but non  
25 necessarily greater than 7, such as for example: sorbitan-esters of fatty acids (e.g. Span®, Arlacel®, Brij®), polyoxyethylen sorbitan esters of fatty acids (e.g. Tween®, Capmul®, Liposorb®), copolymers of polypropyleneoxide-polyethyleneoxide (Poloxamer), esters of polyethylene glycol (PEG)-glycerol (Labrasol®, Labrafil® with HLB 6-7), esters of PEG and acids or long chain  
30 aliphatic alcohols (e.g. Cremophor®), polyglycerid esters (Plurol®), esters of saccharides and fatty acids (sucro-esters). When needed, even anionic

surfactants (e.g. sodium lauryl sulfate, sodium stearate, sodium oleate), bile salts (e.g. sodium glycocholate, taurodeoxycholate, taurocholate, ursodeoxycholate) or cationic (e.g. tricetol), as well as low HLB surfactants, lecithins as they are (Lipoid S75) and hydrogenated (e.g. Lipoid S75, S75-3), phospholipids and their semisynthetic or synthetic derivatives may be used.

Among the cosurfactants needed for the formation of the microemulsion we remember short chain alcohols such as for example ethanol, 2-propanol, n-butanol, isopropanol; short and medium aliphatic acids (e.g. butyric acid, valeric and capronic acids), aromatic alcohols (e.g. benzyl alcohol); medium chain alcohols and aliphatic acids (C<sub>8</sub>-C<sub>12</sub>) such as decanoic acid, lauric acid, caprynil alcohol and lauryl alcohol. Moreover, as cosurfactants may be used also esters or ethers of acids or medium-long chain aliphatic alcohols with mono- or polyhydroxylated alcohols. Some of the components mentioned among the cosurfactants may at the same time form the oil phase of the microemulsion.

The pharmaceutical active principles usable in the invention may be both hydrosoluble (e.g. peptides or proteins) and liposoluble (e.g. steroidal hormones), as well as poorly soluble in both vehicles (e.g. acyclovir). The surface and mass properties of the nanoparticles according to the invention allow important advantages such as for example:

- 1) the possibility of administering by oral or transmucosal way molecules usually not absorbable by such a way (e.g. polypeptides and proteins);
- 2) the possibility of administering by oral and/or parenteral way lipophilic highly insoluble and poorly absorbable molecules;
- 3) an improvement in the biopharmaceutical properties of the active principles (e.g. controlled or prolonged release and increase of the plasmatic half-life time);
- 4) the possibility of administering by topical way molecules active at the mucosal or dermal level (e.g. antiviral, antimicotic, antipsoriatic drugs);
- 5) the possibility of encapsulating active principles having unpleasant flavour, administrable in immediate release formulations.

The active principle groups which may be advantaged from the invention include: non steroidal (NSAID) and steroidal (SAID) anti-inflammatories, estrogenic or

progestational hormones, cardiovasculars, antivirals, antimycotics, antineoplastics, hypolipidemics, peptides and proteins having different action.

Among said active principles we may mention, however as a not exhaustive example:

- 5 ergot alkaloids and derivatives: dihydroergotamine, dihydroergotoxine and bromocriptine.

Analgesics and non steroidal anti-inflammatories, and their salts: diclofenac sodium, diclofenac hydroxyethyl pyrrolidine, diclofenac diethylamine, ibuprofen, flurbiprofen, ketoprofen, indomethacin, mefenamic acid, naproxen, nimesulide and  
10 piroxicam.

Antiarrhythmics: amiodarone, diisopyramide, propranolol and verapamil.

Antibacterials: amoxicillin, flucloxacillin, gentamicin, rifampicin, erythromycin and cephalosporins.

Antifungins and antipsoriatics: amphotericin, butoconazole nitrate, ketoconazole, econazole, etretinate, fluconazole, flucytosine, griseofulvin, itraconazole,  
15 miconazole, nystatin, sulconazole and tioconazole.

Antivirals: Acyclovir, ganciclovir, AZT and protease inhibitors.

Antihypertensives: amlodipine, clonidine, diltiazem, felodipine, guanabenz acetate, isradipine, minoxidil, nicardipine hydrochloride, nimodipine, nifedipine, prazosin  
20 hydrochloride and papaverine.

Antidepressants: carbamazepine.

Antihistaminics: diphenhydramine, chlorpheniramine, pyrilamine, chlorcyclizine, promethazine, acrivastine, cinnarizine, loratadine and terfenadine.

Antineoplastics and immunosuppressants: cyclosporin, dacarbazine, etretinate, etoposide, lomustine, melphalan, mitomycin, mitoxantrone, paclitaxel,  
25 procarbazine, tamoxifen, taxol and derivatives and taxotere.

Anxiolytics, sedatives, hypnotics: alprazolam, bromazepam, diazepam, lorazepam, oxazepam, temazepam, sulpiride and triazolam.

$\beta$ -Blockers: alprenolol, atenolol, oxprenolol, pindolol and propranolol.

30  $\beta$ -Agonists: salbutamol, salmeterol.

Cardiac and cardiovascular inotropics: amrinone, digitoxin, digoxin, lanatoside C,

medigoxin and ubidecarenone.

Corticosteroids: beclomethasone, betamethasone, budesonide, cortisone acetate, desoximethasone, dexamethasone, fludrocortisone acetate, flunisolide, hydrocortisone, methylprednisolone, methylprednisone and triamcinolone.

- 5 Gastrointestinals and anti H2-histaminics: cimetidine, cisapride, domperidone, famotidine, loperamide, mesalazine, omeprazole, ondansetron hydrochloride and ranitidine.

Hypolipidemics: bezafibrate, clofibrate, gemfibrozil, probucol and lovastatin.

- 10 Anti-anginals: amyl nitrate, glyceryl trinitrate, isosorbide dinitrate and mononitrate and pentaerythritol tetranitrate.

Central Action Drugs: for example nicotine.

Vitaminic and Nutritional Agents: betacarotene, vitamin A, Vitamin B2, Vitamin D and derivatives, vitamin E and derivatives and vitamin K.

- 15 Opioid Analgesics: codeine, dextropropoxyphene, dihydrocodeine, morphine, pentazocine and methadone.

Sexual Hormones: danazol, ethinyl estradiol, medroxyprogesterone acetate, methyltestosterone, testosterone, norethistrone, norgestrel, estradiol, estriol, progesterone, stilbestrol and diethylstilbestrol.

Peptidic, proteic or polysaccharidic molecules having different activity:

- 20 leuprolide and LH-RH analogues, calcitonin, glutathione, somatotropin (GH), somatostatin, desmopressin (DDAVP), interferon, molgramostin, epidermic growth factor (EGF), nervous growth factor (NGF), insulin, glucagon, toxins or toxoides (for example tetanus toxin), antigenic factors of proteic or polysaccharidic kind, heparin, heparin having low molecular weight and heparinoids.

- 25 Molecules having specific topical activity: e.g. sun protectors (UV Absorbers); skin nutrients, ceramides and glycolic acid.

The characteristics of the compositions according to the invention may be evaluated by several physico chemical methods, such as for example:

- 30 - thermal analysis (DSC, TGA and "hot stage" microscopy) in order to verify the change of the mass properties,  
- surface analysis (angle of contact method) to determine the variation of the



surface of the surface properties,

- laser light scattering (LLS) techniques for the determination of the size distribution of the nanoparticles,
- Zeta potential measurement techniques for the determination of the superficial charge properties of the nanoparticles,
- active principles incorporation efficiency determination techniques (marking techniques with fluorescent molecules, separation and analysis techniques),
- morphological observation techniques (electron transmission microscopy: TEM).

Such techniques allow to demonstrate that the composite materials forming the

nanoparticles according to the invention and the nanoparticles themselves show innovative and advantageous properties. Using the suitable combinations of lipidic and amphiphilic material, it is possible to prepare materials and composite nanoparticles which may:

- increase the incorporation efficiency of hydrophilic drugs (e.g. peptides) in a lipidic matrix, helping the dissolution/dispersion of the active principle in the most hydrophilic zones of the nanoparticles themselves,
- modify in an unexpected way the localization of the molecules incorporated into the nanoparticles: for example, allowing a homogeneous distribution, inside the lipophilic nanoparticles, of peptidic molecules usually adsorbed on the surface,
- show a surface energy lower with respect to the starting components, thus resulting more biocompatible,
- show surfaces more lipophilic than the single components, and thus result more absorbable by oral way,
- when useful to the application, show surfaces more hydrophilic than the single components and increase the plasmatic half-life times of the particles injected by parenteral way,
- at certain proportions of amphiphilic and lipidic substance, result in two phases, with peculiar characteristics, wherein the amphiphilic compound:
  - 1) may be preferentially located on the surface of the nanoparticles ("segregated in surface");
  - 2) may be preferentially located inside the nanoparticles ("segregated inside");

3) may be homogeneously located on the surface and inside the nanospheres themselves,

- at certain proportions the nanoparticles, consisting of composite materials having peculiar and different characteristics with respect to the single original materials, may melt at physiological temperatures releasing the active principle in a fast way (e.g. for topical/percutaneous treatments and flavour masking),

- at other proportions, the nanoparticles may stay as they are at physiological temperatures ensuring the release of the active principle by diffusion and/or degradation of the nanoparticles themselves,

- at certain proportions of lipidic and amphiphilic material, in the preparation technique (1), based on the oil-water microemulsion of the composite mixture, it is possible to extend the thermal interval of existence of the microemulsion itself to lower temperature values, allowing the incorporation of thermolabile molecules.

Fundamental and innovative characteristic of the invention, independently from the process of preparation of the nanoparticles, is thus the unexpected formation of a composite material having new and different surface and mass characteristics with respect to the single components. On the base of the qualitative characteristics of the chosen starting materials (e.g. chemical structure, melting point, hydrophobicity and wettability), and at the relative percentages of the materials in a determined mixture, it is possible to obtain a very high number of composite materials, and thus of nanoparticles having different characteristics.

Advantage of the invention is the possibility to increase the incorporation of the hydrophilic active principle inside a lipophilic nanoparticle, and to be able to modify its distribution.

An important advantage of the invention thus consists in the possibility to have vector systems (composite nanoparticles) consisting of new materials originated by exclusively physical changes of the component substances and thus not requesting the long toxicological experimental tests iter.

### EXAMPLES

For illustrative aim examples of preparation of the compositions according to the invention are reported, with the technique 1 (Examples 1-36), and with the

technique 2 (Examples 37-45), and Comparative Examples with preparations according to the prior art (A-E). The characterization of the obtained products and the advantageous behaviour in vivo with respect to the products of the prior art are reported in the Examples (F-Z 1).

#### 5 EXAMPLE 1

4 grams of stearic acid and 19.9 mg of di-myristoil-phosphatidyl glycerol (DMPG percentage in the mixture: 0.5%) are mixed, and they are heated to about 72 °C with the formation of the melted composite mixture to which 4 ml of n-butanol are added. In 20 ml of water acidified to pH 3, 2 g of sodium taurodeoxycholate and 10 3.85 mg of salmon calcitonin are dissolved and the solution heated to 72 °C is added to the melted composite mixture. To the so formed emulsion, kept under stirring, 3 ml of Tween 20 are added, to form a transparent and anisotropic microemulsion. The microemulsion, at about 60 °C, is dispersed in 5 volumes of water at pH 3 cooled to 3-5 °C, under constant stirring (250 rpm), to form 15 composite solid lipidic nanoparticles consisting of stearic acid - dimyristoil-phosphatidyl glycerol containing calcitonin. The suspension is washed by ultrafiltration in order to remove the surfactant excess. The calcitonin incorporation efficacy in the nanoparticles, determined by fluorimetric and chromatographic techniques (see characterization Examples), is about 10.5 %. The average 20 diameter of the nanoparticles is 226 nm and the polydispersion index is 0.200.

#### EXAMPLE 2

The preparation of the Example 1 is repeated, using a DMPG percentage in the composite mixture equal to 1.0%. The calcitonin incorporation efficacy is 10.2%, the average diameter of the nanoparticles 220 nm and the polydispersion 0.268.

#### 25 EXAMPLE 3

The preparation of the Example 1 is repeated, using a DMPG percentage in the composite mixture equal to 4.5%. The calcitonin incorporation efficacy is 13.2%, the average diameter of the nanoparticles 199 nm and the polydispersion 0.212.

#### EXAMPLE 4

30 The preparation of the Example 1 is repeated, using a DMPG percentage in the composite mixture equal to 5.2%. The calcitonin incorporation efficacy is 13.4%,

the average diameter of the nanoparticles 195 nm and the polydispersion 0.200.

#### EXAMPLE 5

The preparation of the Example 1 is repeated, using a DMPG percentage in the composite mixture equal to 9.5%. The calcitonin incorporation efficacy is 9.19%,  
5 the average diameter of the nanoparticles 231 nm and the polydispersion 0.186.

#### EXAMPLE 6

The preparation of the Example 1 is repeated, using a DMPG percentage in the composite mixture equal to 25%. The calcitonin incorporation efficacy is 9.51%,  
the average diameter of the nanoparticles 205 nm and the polydispersion 0.275.

#### EXAMPLE 7

The preparation of the Example 1 is repeated, using as an amphiphilic material the distearoil phosphatidic acid (DSPA) phospholipid in a percentage in the composite mixture equal to 1.2%. The calcitonin incorporation efficacy is 9.9%, the  
average diameter of the nanoparticles 245 nm and the polydispersion 0.261.

#### EXAMPLE 8

The preparation of the Example 7 is repeated, using a DSPA percentage in the composite mixture equal to 5.2%. The calcitonin incorporation efficacy is 14.4%,  
the average diameter of the nanoparticles 324 nm and the polydispersion 0.341.

#### EXAMPLE 9

The preparation of the Example 1 is repeated, using low molecular weight heparin as an active principle (average molecular weight 4000 Da) and, the dimyristoil phosphatidylcholine (DMPC) phospholipid as amphiphilic material, in a percentage in the composite mixture equal to 4.0%. The heparin incorporation efficacy is about 1.0%, the average diameter of the nanoparticles is 213 nm and the  
25 polydispersion 0.186.

#### EXAMPLE 10

The preparation of the Example 9 is repeated, using the dimyristoil phosphatidylcholine (DMPC) phospholipid, in a percentage in the composite mixture equal to 8.0%. The average diameter of the nanoparticles is 179 nm and  
30 the polydispersion 0.249.

#### EXAMPLE 11

The preparation of the Example 9 is repeated, using the dimyristoil phosphatidylcholine (DMPC) phospholipid, in a percentage in the composite mixture equal to 10%. The average diameter of the nanoparticles is 290 nm and the polydispersion 0.270.

5 EXAMPLE 12

The preparation of the Example 9 is repeated, using the dimyristoil phosphatidylcholine (DMPC) phospholipid, in a percentage in the composite mixture equal to 14%. The average diameter of the nanoparticles is 369 nm and the polydispersion 0.360.

10 EXAMPLE 13

The preparation of the Example 1 is repeated, using the Labrafac Hydro™ polyhydroxylated triglyceride as amphiphilic material, in a percentage in the composite mixture equal to 1.0%. The average diameter of the nanoparticles is 307 nm and the polydispersion 0.234.

15 EXAMPLE 14

The preparation of the Example 13 is repeated, using the Labrafac Hydro™ polyhydroxylated triglyceride, in a percentage in the composite mixture equal to 2.5%. The average diameter of the nanoparticles is 307 nm and the polydispersion 0.234.

20 EXAMPLE 15

The preparation of the Example 13 is repeated, using the Labrafac Hydro™ polyhydroxylated triglyceride, in a percentage in the composite mixture equal to 50%. The average diameter of the lipidic nanoparticles is 310 nm and the polydispersion 0.332.

25 EXAMPLE 16

The preparation of the Example 13 is repeated, using the Labrafac Hydro™ polyhydroxylated triglyceride, in a percentage in the composite mixture equal to 10.0%. The average diameter of the nanoparticles is 326 nm and the polydispersion 0.325.

30 EXAMPLE 17

The preparation of the Example 13 is repeated, using the Labrafac Hydro™

polyhydroxylated triglyceride, in a percentage in the composite mixture equal to 10.0% and incorporating in the composite mixture 3.87 mg of calcitonin in 3.8 g of the mixture itself. The average diameter of the nanoparticles is 326 nm and the polydispersion 0.325.

5 EXAMPLE 18

2.18 g of stearic acid and 67 mg of Labrafil™ M 2130CS polyhydroxylated triglyceride (Labrafil percentage in the composite mixture: 3.1%) are mixed, and they are heated to about 75 °C with the formation of the melted composite mixture. The mixture is solidified by cooling. In 10 ml of water, acidified to pH 3,  
10 1.34 g of sodium taurodeoxycholate and 3.85 mg of salmon calcitonin are dissolved, the solution is heated to 72 °C and it is added to the melted composite mixture at about 72 °C, to which 1 ml of n-butanol had been added. To the so formed emulsion kept under stirring, 4 ml of Tween 20 are added, to form a transparent and anisotropic microemulsion. The microemulsion, heated to about  
15 50 °C is dispersed in 50 volumes of water at pH 3 cooled to about 3-5 °C, under constant stirring (250 rpm), to form the solid nanoparticles containing calcitonin. The suspension is washed by ultrafiltration in order to remove the surfactant excess. The calcitonin incorporation efficacy in the nanoparticles is about 11.5 %. The average diameter of the nanoparticles is 185 nm and the polydispersion index  
20 is 0.300.

EXAMPLE 19

The preparation of the Example 18 is repeated without the incorporation of calcitonin. The average diameter of the composite nanoparticles is 182 nm and the polydispersion index 0.295.

25 EXAMPLE 20

The preparation of the Example 18 is repeated, using the Labrafil 2130CS polyhydroxylated triglyceride at 8.9% in the composite mixture. The composite mixture is melted at about 70 °C. The average diameter of the nanoparticles is 173 nm and the polydispersion index 0.268.

30 EXAMPLE 21

The preparation of the Example 18 is repeated, using the Labrafil 2130CS

polyhydroxylated triglyceride at 10% in the composite mixture. The composite mixture is melted at 66 °C. The average diameter of the nanoparticles is 216 nm and the polydispersion index 0.287.

#### EXAMPLE 22

- 5 The preparation of the Example 18 is repeated, using the Labrafil 2130CS polyhydroxylated triglyceride at 15% in the composite mixture. The average diameter of the nanoparticles is 188 nm and the polydispersion index 0.247.

#### EXAMPLE 23

- 10 The preparation of the Example 18 is repeated, using the Labrafil 2130CS polyhydroxylated triglyceride at 50% in the composite mixture and the composite mixture is melted at 60 °C. The average diameter of the nanoparticles is 312 nm and the polydispersion index 0.424.

#### EXAMPLE 24

- 15 The preparation of the Example 18 is repeated, using the Labrafil 2130CS polyhydroxylated triglyceride at 75% in the composite mixture and the composite mixture is melted at 54 °C. The average diameter of the nanoparticles is 162 nm and the polydispersion index 0.315.

#### EXAMPLE 25

- 20 The preparation of the Example 18 is repeated, using the Labrafil 2130CS polyhydroxylated triglyceride at 95% in the composite mixture and the composite mixture is melted at 35 °C. The average diameter of the nanoparticles is 205 nm and the polydispersion index 0.281.

#### EXAMPLE 26

- 25 1.5 g of stearic acid and 0.5 g of decanoic acid (decanoic acid percentage in the mixture: 25%) are mixed, and the mixture is heated to about 75 °C with the formation of the composite mixture. The mixture is solidified by cooling. In 10 ml of water acidified to pH 3 1.30 g of sodium taurodeoxycholate are dissolved and the solution heated to 55 °C is added to the melted composite mixture at about 55 °C, to which 1 ml of n-butanol had been added. To the so formed emulsion, kept  
30 under stirring, 2.6 ml of Tween 20 are added to form a transparent and anisotropic microemulsion. The microemulsion maintained at about 45 °C is dispersed in 50

volumes of water at pH 3 cooled to about 3-5 °C, under constant stirring (250 rpm), to form low melting solid nanoparticles. The suspension is washed by ultrafiltration in order to remove the surfactant excess. The average diameter of the nanoparticles is 280 nm.

5 EXAMPLE 27

The preparation of the Example 26 is repeated, using the decanoic acid at 50% in the composite mixture. The composite mixture is melted at 50 °C. The average diameter of the nanoparticles is 310 nm and the polydispersion index 0.280.

EXAMPLE 28

10 The preparation of the Example 26 is repeated, using the decanoic acid at 75% in the composite mixture. The composite mixture is melted at 35 °C. The average diameter of the nanoparticles is 300 nm and the polydispersion index 0.250.

EXAMPLE 29

15 1.8 g of stearic acid and 0.2 g of polyethylene glycol PEG 4000 (PEG 4000 percentage in the mixture: 10.0%) are mixed, and they are heated to about 75 °C with the formation of the composite mixture. In 10 ml of water, acidified to pH 3 1.30 g of sodium taurodeoxycholate are dissolved and the solution heated to 50 °C is added to the melted composite mixture at about 50 °C, to which 0.5 ml of n-butanol had been added. To the so formed emulsion, kept under stirring, 2.4 ml of  
20 Tween 20 are added to form a transparent and anisotropic microemulsion. The microemulsion maintained at about 45 °C is dispersed in 50 volumes of water at pH 3, at about 3-5 °C, under constant stirring (250 rpm), to form the composite lipidic solid nanoparticles. The suspension is washed by ultrafiltration in order to remove the surfactant excess. The average diameter of the nanoparticles is 184  
25 nm and the polydispersion 0.302.

EXAMPLE 30

The preparation of the Example 29 is repeated using the polyethyleneglycol PEG 4000 at 20% in the composite mixture. The composite mixture is melted at 50 °C. The average diameter of the nanoparticles is 263 nm and the polydispersion index  
30 0.334.

EXAMPLE 31



The preparation of the Example 29 is repeated, using the poly-(propyleneoxide) poly-(ethyleneoxide) copolymer as amphiphilic material, Lutrol™ 188 at 10% in the composite mixture. The composite mixture is melted at 50 °C. The average diameter of the nanoparticles is 353 nm and the polydispersion index 0.314.

#### 5 EXAMPLE 32

The preparation of the Example 29 is repeated, using Lutrol™ 188 at 20% in the composite mixture. The composite mixture is melted at 50 °C. The average diameter of the nanoparticles is 375 nm and the polydispersion index 0.300.

#### EXAMPLE 33

10 1.4 g of stearic acid and 607 mg of Labrafil™ M2130CS polyhydroxylated triglyceride (Labrafil percentage in the mixture: 30.1%) are mixed, and they are heated to about 70 °C with the formation of the composite mixture. To such a mixture 1.06 g of partially hydrogenated soybean lecithin (Lipoid S75-35) are added. 10 ml of an aqueous solution at pH 3 are added to the composite mixture  
15 melted at about 70 °C, to which 10 ml of n-butanol has been added. To the so formed emulsion, kept under stirring, 6 ml of Tween 20 are added to form a transparent and anisotropic microemulsion. In the microemulsion about 200 mg of ciclosporin are added. The microemulsion, heated to about 50 °C is dispersed in 50 volumes of water at pH 3, cooled to about 3-5 °C, under constant stirring (250  
20 rpm), to form the solid nanoparticles containing ciclosporin. The suspension is washed by ultrafiltration in order to remove the surfactant excess. The average diameter of the nanoparticles containing ciclosporin is 304 nm and the polydispersion index 0.365.

#### EXAMPLE 34

25 The preparation of the Example 33 is repeated, incorporating in the microemulsion 100 mg of etoposide. The composite mixture is melted at 50 °C. The average diameter of the composite nanoparticles is 288 nm and the polydispersion 0.211.

#### EXAMPLE 35

30 The preparation of the Example 29 is repeated, using Lutrol™ 188 at 20% in the composite mixture. The composite mixture is melted at 50 °C and added with acyclovir in an amount equal to 100 mg per gram of composite mixture. The

average diameter of the nanoparticles containing acyclovir is 360 nm and the polydispersion index 0.302.

#### EXAMPLE 36

980 mg of stearic acid and 510 mg of Labrafil™ M2130CS polyhydroxylated triglyceride (Labrafil percentage in the mixture: 30%) are mixed, and they are heated to about 70 °C with the formation of the composite mixture. To such a mixture 300 mg of Q10 coenzyme (Ubidecarenone) and 1.06 g of partially hydrogenated soybean lecithin (Lipoid S75-35) are added. 10 ml of an aqueous solution at pH 3 are added to the composite mixture melted at about 70 °C, to which 1 ml of n-butanol has been added. To the so formed emulsion, kept under stirring, 6 ml of Tween 20 are added to form a transparent and anisotropic microemulsion. The microemulsion, heated to about 50 °C is dispersed in 50 volumes of water at pH 3, at about 3-5 °C, under constant stirring (250 rpm), to form the solid nanoparticles containing ubidecarenone. The suspension is washed by ultrafiltration in order to remove the surfactant excess. The ubidecarenone incorporation percentage in the nanoparticles is 99%, the average diameter of the nanoparticles is 195 nm and the polydispersion index 0.214.

#### EXAMPLE 37

6 g of stearic acid and 0.9 g of Labrafil™ M2130CS polyhydroxylated triglyceride (15% of the mixture) are mixed, which are melted at a temperature about equal to 70 °C. To the melted composite mixture 1.5 g of soybean lecithin (Lipoid S75-35) are added, and 300 ml of an aqueous solution at pH 5.5 containing 3 g of Tween 20. The so formed emulsion is passed in a high pressure Rannie-MiniLab 8.30 homogenizer, at a temperature equal to 70 °C and a pressure equal to 750 bar for times ranging from 0 to 15 min. The dispersions are recovered and instantaneously cooled to 4 °C, by constant stirring at 250 rpm in a thermostated bath, giving origin to solid nanoparticles. The average diameters are:

Process Time (min)	Diameter (nm)	Polydispersion
5	257	0.36
10	264	0.392
15	282	0.400

**EXAMPLE 38.**

The preparation of the Example 37 is repeated, maintaining the temperature of the system, once prepared the composite material by comelting, below the melting temperature of the composite itself ( $T < 50\text{ }^{\circ}\text{C}$ ). The obtained dispersion is pre-homogenized for 5 minutes in a low energy (Silverson mod. L2R) homogenizer-mixer, and subsequently homogenized at high pressure (750 bar) at constant  $T^{\circ}$  ( $45\text{ }^{\circ}\text{C}$ ).

**EXAMPLE 39.**

0.39 g of stearic acid and 5.5 g of Labrafil™ M2130CS polyhydroxylated triglyceride (95% of the mixture) are mixed, which are melted to a temperature equal to  $75\text{ }^{\circ}\text{C}$ . To the melted composite mixture 3 g of soybean lecithin (Lipoid S75-35) are added and 300 ml of an aqueous solution at pH 5.5 containing 12 g of Tween 20. The so formed emulsion is passed in a high pressure Rannie-MiniLab 8.30 homogenizer at a temperature equal to  $70\text{ }^{\circ}\text{C}$  and a pressure equal to 750 bar for times ranging from 0 to 15 min. The dispersions are instantaneously cooled to  $4\text{ }^{\circ}\text{C}$  by constant stirring at 250 rpm in a thermostated bath, giving origin to composite lipidic solid nanoparticles. The average diameters are:

Process Time (min)	Diameter (nm)	Polydispersion
3	106	0.205
5	72	0.161
10	91	0.176

**EXAMPLE 40**

The preparation of the Example 39 is repeated, maintaining the temperature of the system, once prepared the composite material by comelting, under the melting temperature of the composite itself ( $T < 35\text{ }^{\circ}\text{C}$ ). The obtained dispersion is pre-homogenized in a low energy (Silverson mod. L2R) homogenizer-mixer, and subsequently homogenized at high pressure (750 bar) at constant  $T$  ( $30\text{ }^{\circ}\text{C}$ ).

#### EXAMPLE 41

3.75 g of stearic acid and 3.75 g of polyethylene glycol 20000 (PEG 20000) (50% in the mixture) are mixed, which are melted to a temperature equal to  $75\text{ }^{\circ}\text{C}$ . To the melted composite mixture 3 g of soybean lecithin (Lipoid S75-35) are added and 300 ml of an aqueous solution at pH 5.5 containing 6 g of Tween 20. The so formed emulsion is passed in a high pressure Rannie-MiniLab 8.30 homogenizer, at a temperature equal to  $70\text{ }^{\circ}\text{C}$  and a pressure equal to 750 bar for times ranging from 1 to 10 min. The dispersions are instantaneously cooled to  $4\text{ }^{\circ}\text{C}$  by constant stirring at 250 rpm in a thermostated bath, giving origin to solid nanoparticles. The average diameters are:

Process Time (min)	Diameter (nm)	Polydispersion
5	156	0.274
10	188	0.308

#### EXAMPLE 42

The preparation of the Example 41 is repeated, maintaining the temperature of the system, once prepared the composite material by comelting, under the melting temperature of the composite itself ( $T < 45\text{ }^{\circ}\text{C}$ ). The obtained dispersion is pre-homogenized in a low energy (Silverson mod. L2R) homogenizer-mixer, and subsequently homogenized at high pressure (750 bar) at constant  $T$  ( $45\text{ }^{\circ}\text{C}$ ).

#### EXAMPLE 43

The preparation of the Example 41 is repeated, with a PEG percentage equal to 15% in the composite mixture and incorporating in the composite mixture itself 300 mg of salmon calcitonin. The efficiency of the calcitonin incorporation in the PEG-stearic acid mixture is equal to 35%.

EXAMPLE 44

The preparation of the Example 42 is repeated maintaining the temperature of the system, once prepared the composite material by comelting below the melting temperature of the composite itself ( $T < 45\text{ }^{\circ}\text{C}$ ). The obtained dispersion is pre-homogenized in Silverson and subsequently homogenized at high pressure (750 bar) at constant  $T$  ( $45\text{ }^{\circ}\text{C}$ ).

EXAMPLE 45

A composite mixture containing 5% stearic acid and 95% Labrafil™ M2130CS polyhydroxylated triglyceride is prepared by comelting and cooling. To the melted composite mixture ( $75\text{ }^{\circ}\text{C}$ ) 1 g of ibuprofen and 240 mg per gram of mixture of soybean lecithin (Lipoid S75-35) and an aqueous solution containing 1.2% of Tween 20 in an amount of 40 ml of aqueous solution at pH 5.5 per gram of composite mixture + drug are added. The so formed emulsion is treated in a high pressure Rannie-MiniLab 8.30 homogenizer, at a temperature equal to  $70\text{ }^{\circ}\text{C}$  and a pressure equal to 750 bar, for times ranging from 0 to 10 min. The dispersions are instantaneously cooled to  $4\text{ }^{\circ}\text{C}$  by constant stirring at 250 rpm in a thermostated bath, giving origin to solid nanoparticles. The average diameters are:

Process Time (min)	Diameter (nm)	Polydispersion
2	224	0.304
5	264	0.364
10	253	0.352

EXAMPLE A (ACCORDING TO THE PRIOR ART EP 0526666A1)

4.2 grams of stearic acid are melt heated to about  $72\text{ }^{\circ}\text{C}$ . In 20 ml of water acidified, at pH 3, 2.6 g of sodium taurodeoxycholate are dissolved and the solution, warmed to  $72\text{ }^{\circ}\text{C}$  is added to the melted stearic acid, to which 2 ml of n-butanol have been added. To the so formed emulsion, maintained under stirring in a mixer, 5 ml of Tween 20 are added obtaining a transparent and anisotropic microemulsion. The microemulsion, taken to about  $60\text{ }^{\circ}\text{C}$ , is dispersed in 5 volumes of water at pH 3, at about  $3\text{--}5\text{ }^{\circ}\text{C}$ , under constant stirring (250 rpm), to

form the nanoparticles according to the technique used in EP 0526666A1. The suspension is washed by ultrafiltration to remove the surfactant excess. The average diameter of the nanoparticles is 209 nm and the polydispersion index is 0.155.

5 EXAMPLE B

The procedure of the Example A is repeated, adding 3.85 mg of salmon calcitonin. The incorporation efficacy of the calcitonin in the nanoparticles is 1.82%. The average diameter of the nanoparticles is 193 nm and the polydispersion index is 0.235.

10 EXAMPLE C

The procedure of the Example B is repeated. To the so prepared nanoparticles an amount of amphiphilic material (dimyristoil phosphatidyl glycerol, DMPG) is added, under stirring in a mixer, in a ratio equal to 1:10 with respect to the lipidic mass of the stearic acid, which is adsorbed on the surface of the nanoparticles themselves. The incorporation efficacy of the calcitonin on the nanoparticles is equal to 1.75%. Such composition according to the prior art is directly comparable with the Example 5 of the invention, deferring only for having the adsorbed amphiphilic component on the surface and not as a component of the composite material forming the nanoparticles. The suspension is washed by ultrafiltration in order to remove the surfactant excess. The average diameter of the nanoparticles is 215 nm and the polydispersion index is 0.175.

EXAMPLE D

1.7 g of stearic acid and 300 mg of ubidecarenone are mixed and they are melt heated to about 70 °C. 0.5 ml of n-butanol, 1.30 g of sodium taurodeoxycholate in 10 ml of aqueous solution at pH 3, heated to 70 °C are added to the comelted. To the so formed emulsion, maintained under stirring, 3.25 g of Tween 20 are added to form a microemulsion. The microemulsion heated to about 70 °C is dispersed in 50 volumes of water at pH 3 at about 3-5 °C under constant stirring (250 rpm), to form the lipidic nanoparticles according to the EP 0526666A1 technique. The suspension is washed by ultrafiltration to remove the surfactant excess. The incorporation percentage of ubidecarenone in the nanoparticles is about 80%, the

average diameter of the nanoparticles is 205 nm and the polydispersion index is 0.244.

EXAMPLE E (ACCORDING TO THE PRIOR ART WO 93/05768)

7.5 g of stearic acid are melted at a temperature equal to 75 °C. 3 g of soybean lecithin (Lipoid S75-35) are added and 300 ml of aqueous solution at pH 5.5 containing 6 g of Tween 20. The so obtained emulsion is treated in a high pressure Rannie-MiniLab 8.30 homogenizer at a temperature equal to 70 °C and a pressure equal to 750 bar, for times ranging from 0 to 10 min. The dispersions are instantaneously cooled to 4 °C giving origin to solid nanoparticles according to the technique described in WO 93/05768.

The average diameters are:

Process Time (min)	Diameter (nm)	Polydispersion
5	190	0.254
10	178	0.308

CHARACTERIZATION EXAMPLES

**15 - Improvement of the incorporation characteristics of hydrophilic drugs (peptides)**

One of the innovative characteristics of the invention is the possibility to improve the efficacy of the incorporation of hydrophilic drugs (peptides) and to improve the distribution inside the composite nanoparticles. Such aspect has been shown by the fluorescence techniques.

The calcitonin peptide has been marked by a fluorophor (7-nitrobenz-2oxa-1,3diazol, NBD), according to a known technique (Biochem. J., 272, 713-719, [1990]), and subsequently incorporated in the nanoparticles as described in the Examples 1-8 of the invention and the Comparative Examples B-C. The samples have been washed according to the ultrafiltration procedures described in the Examples.

- The percentage of peptide incorporated in the nanoparticles

• The percentage of peptide superficially adsorbed, with respect to the incorporated total, measuring the fluorescence before and after the treatment of the suspensions with the proteolytic enzyme tripsine, able to dissolve and degradate only the peptide fraction adsorbed on the surface of the particles themselves

have been calculated, by the measure of the emission values in fluorescence with respect to a standard curve, and with respect to 100% of fluorescence emitted before the ultrafiltration.

The results, reported in the Example F, Table 1, show how the composite nanoparticles increase the incorporation efficacy of the peptide, decreasing its superficially located fraction (adsorbed and attackable by the proteolytic enzymes), and maintaining the majority inside the composite matrix.



**EXAMPLE F**

**Table 1.** The calcitonin incorporation efficiency in composite lipidic nanoparticles, and percentage of peptide adsorbed on the surface of the particles themselves.

Example	Materials	Composition (%)	Incorporation Efficiency (%)	Adsorbed Peptide (%)
1	Stearic Acid DMPG	99.5 0.5	10.5	n.d.
2	Stearic Acid DMPG	99.0 1.0	10.2	0.11
3	Stearic Acid DMPG	95.5 4.5	13.2	0.05
4	Stearic Acid DMPG	94.8 5.2	13.4	0.04
5	Stearic Acid DMPG	91.5 9.5	9.19	0.49
6	Stearic Acid DMPG	75 25	9.51	3.98
7	Stearic Acid DSPA	98.8 1.2	9.9	n.d.
8	Stearic Acid DSPA	94.8 5.2	14.4	n.d.
43	Stearic Acid PEG	85 15	35	n.d.
B	Stearic Acid	100	1.82	0.93
C	Stearic Acid DMPG	90.0 10.0	1.75	0.95

5 - Modification of the mass characteristics

Another innovative characteristic of the invention product is the modification of the mass properties of the composite materials forming the nanoparticles. The modifications of the mass properties are quantitatively evaluated by thermal analysis techniques by a differential scanning calorimeter, DSC, Perkin Elmer mod. DSC 7. The nanoparticles according to the invention may be formed either by an homogenous composite material, or by a composite material presenting separated phases with different properties. In the former case it is possible for example to observe that the homogeneous composite material shows the modification of a thermal event (melting temperature) as a function of the composition (Example G) (Table 2). In the latter case more thermal events, related to the thermal transitions or to the melting of separated phases, occurring at temperatures and with specific transition enthalpies for each per cent composition (Examples H, I) (Tables 3 and 4) are distinguishable. In both cases, the characteristics of the composite material are unexpected and unforeseeable on the base of the single materials.

The modification of the thermodynamical behaviour of the composite nanoparticles as a function of the component percentage is observable also on the nanoparticles in suspension, for example by techniques of laser light scattering ("laser light scattering"). The phase transitions of the composite nanoparticles are noticeable from the intensity variation of the scattering as a function of the temperature, measured by a Brookhaven mod. BI-90 Particle Sizer. By this technique, it is possible to point out how the composite nanoparticles show phase transitions and melting at temperatures different according to the composition of the forming material; see: Example L, Figure 1.

**EXAMPLE G**

**Table 2.** Melting point (°C) of binary composite mixtures of stearic acid and average chain fatty acids (amphiphilic) corresponding in part to the Examples 26-28

Amphiphilic %	0	10	20	30	50	65	70	75	85	90	100
---------------	---	----	----	----	----	----	----	----	----	----	-----

Decanoic Acid	71		60	56	50	43		35	28	26	32
Lauric Acid	71	69		62	56		44		43	42	45
Myristic Acid	71	68		64	56		50		51	53	54
Palmitic Acid	71	68		66	60		56		60	61	63

**EXAMPLE H**

**Table 3.** Phase transitions and enthalpies of the composite material stearic acid-dimyristoil-phosphatidyl glycerol (DMPG), and some significative compositions, corresponding in part to the Examples 1-6.

	T <sub>1</sub> (°C)	ΔH (J/g)	T <sub>f</sub> (°C)	ΔH (J/g)
DMPG %				
0	-	-	73.3	210.5
6	55.3	0.55	72.2	185.95
12	55.0	5.811	71.8	160.02
18	55.2	6.71	71.5	146.09
30	56.2	nd	70.2	nd
40	56.4	nd	68.8	nd
50	57.2	35.15	69.7	56.9
100	-	-	125.21	nd

EXAMPLE I

Table 4. Phase transitions and enthalpies of the composite material stearic acid-Labrafil 2130 CS polyhydroxylated triglyceride, corresponding to the Examples 18-25

	$T_1$ (°C)	$\Delta H$ (J/g)	$T_f$ (°C)	$\Delta H$ (J/g)
Labrafil %				
0	-	-	73.9	210.5
2	24.	0.01	74.1	210.2
5	25.1	0.4	73.3	204.4
10	25.2	1.5	71.6	189.8
15	25.1	2.5	72.0	180.5
50	25.3	6.3	65.4	99.3
78	25.0	8.4	56.0	25.0
85	26.0	8.5	55.1	15.2
90	27.1	10.2	52.0	5.2
100	29.1	13.8	38.6	0.77

EXAMPLE I

Figure 1. Phase transitions of the composite nanoparticles (stearic acid-DMPG), determined by the scattering techniques, corresponding to the described products in the Examples 3-5 and the Comparative Example A. The phase transition (melting) is pointed out by the decrease of the intensity of the laser light scattering ("scattering"), as a function of the temperature. The beginning of such a variation (withdrawal from the curve plateau) corresponds to the beginning of the transition. It is clear how, in the compositions according to the invention, such a transition may be changed and controlled varying the composition of the material forming the nanoparticles. The melting temperature of the particles (63 °C in the Example

A), in fact decreases to 55 °C, 53 °C and 49 °C in the Examples 3, 4, 5 respectively.

**- Modification of the preparation characteristics (technique 1): interval of microemulsion existence**

- 5 The properties of the composite materials according to the invention allow both to obtain compositions having low melting point and to extend the existence thermal interval of the microemulsion, used as intermediate in the preparation of the composite nanoparticles according to the technique 1 (Example M). (Figure 2), at temperatures very much lower than the melting temperature of the single
- 10 component materials. Such interval has been determined by measuring of the scattering frequency, which is minimum ( $< 10$  KHz) in presence of a microemulsion. Evident and principal advantage of the invention is to be able to formulate thermolabile active principles in the composite nanoparticles; a further advantage is to be able to use, for the preparation of the nanoparticles, some
- 15 materials having temperatures lower than their melting temperature.

**EXAMPLE M**

**Figure 2.** Thermal interval of existence of the microemulsion consisting, in oil phase, of stearic acid and decanoic acid (preparative technique 1. See Examples 26-28 and Example A).

20 **- Modification of the surface characteristics**

- One of the innovative characteristics of the product of the invention is the possibility to control the surface properties of the composite materials forming the nanoparticles and, consequently, of the nanoparticles themselves. The surface characteristics of the nanoparticles obtained according to the techniques and the
- 25 materials of the invention differ in substantial way with respect to the surface adsorption of amphiphilic components described in the state of art, because they are dependent from the formation of the composite material. Thus an advantage of the invention is the possibility to obtain composite nanoparticles having favourable characteristics of biocompatibility, hydrophobicity, hydrophilicity and
- 30 polarity according to the therapeutical aim to achieve.

The surface properties of the composite materials forming the nanoparticles may

be measured by the angle of contact method, using a Lorentzen & Wettre apparatus. Such a method allows to calculate the surface energy of the materials, correlable to the wettability, through the equation (1),

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} (\cos \theta) \quad (1)$$

wherein the indexes sv, sl, lv, refer respectively to the surface energy ( $\gamma$ ) of the solid-vapour, solid-liquid and liquid-vapour surfaces, and  $\theta$  is the angle of contact of the liquid with the solid surface. As the superficial free energy of each material may be divided into a polar component and a dispersion component, according to (2):

$$\gamma^t = \gamma^p + \gamma^d \quad (2)$$

it is possible to calculate, from experimental measures elaborated by a series of equations, the values of such components and of the total free energy, as well as the polarity of the surfaces, expressed in percentage as:

$$\%P = (\gamma^p / \gamma^t) * 100 \quad (3)$$

From the surface energy measures of the composite materials according to the invention it has been possible to determine that the amphiphilic component may:

- 1) be preferentially arranged on the surface of the nanoparticles formed by the composite material ("segregated on the surface");
- 2) be preferentially arranged inside the nanoparticles formed by the composite material ("segregated inside");
- 3) be arranged homogeneously on the surface and inside the nanospheres themselves.

The diagrams of the surface energies of the composite mixtures: stearic acid-DMPG (Example N), forming the nanoparticles described in the Examples 1-6, stearic acid-Labrafac Hydro™ polyhydroxylated triglyceride (Example O), forming the nanoparticles described in the Examples 13-17, stearic acid-Labrafac Lipo caprillic-capric triglyceride (Example P) are reported for exemplificative aim in the Figures 3, 4 and 5. In the Figures 3, 4 and 5 the curves A, B and C refer respectively to the total surface free energy, to the dispersion component and to the polar component.

EXAMPLE N

Figure 3. Trend of the surface energy of the stearic acid-DMPG composition, referable to the Examples 1-6. The trend, characterized by a decrease of the total energy, shows a "preferential surface segregation" of the amphiphilic component DMPG in the composite mixture.

EXAMPLE O

Figure 4. Trend of the surface energy of the stearic acid-Labrafac Hydro composition, referable to the Examples 13-17. The trend shows a "preferential inside segregation" of the amphiphilic component Labrafac Hydro in the composite mixture (and of the stearic acid component inside).

EXAMPLE P

Figure 5. Trend of the surface energy of the stearic acid-Labrafac Lipo composition. The invariant trend shows a homogeneous distribution of the amphiphilic component Labrafac Lipo in the composite mixture.

The variation of the surface properties of the composite nanoparticles as a function of the used composite materials composition, is quantifiable also by Zeta potential measures carried out by a laser light scattering electrophoretic analyzer, ZetaMaster (Malvern, UK), on the aqueous suspensions of the composite nanoparticles. The Zeta potential (shear plane potential) is a measure of the surface charge of the nanoparticles in suspension. It is clear from the Examples Q-T that the Zeta potential of the nanoparticles, and consequently the surface properties, range as a function of the amphiphilic material percentage in the composite, with a trend proper to each composition.

The possibility to modify the surface properties and the Zeta potential of the nanoparticles of the invention has moreover the important advantage to decrease or remove the sedimentation or the aggregation trend of the suspensions, increasing then the stability. Such advantage may be then obtained modifying the composition of the material forming the nanoparticles themselves.

TABLE 5. (EXAMPLES Q-T)EXAMPLE Q

Zeta potential (mV) measured on the stearic acid-DMPG nanoparticles, prepared

analogously as the Examples 2-5 and A.

EXAMPLE R

Zeta potential (mV) measured on the stearic acid-DMPG nanoparticles, prepared analogously as the Examples 9-11 and A.

5 EXAMPLE S

Zeta potential (mV) measured on the stearic acid-Labrafac Hydro polyhydroxylated triglyceride nanoparticles, prepared analogously as the Examples 13-16 and A.

EXAMPLE T

10 Zeta potential (mV) measured on the stearic acid-Labrafil 2130CS polyhydroxylated triglyceride nanoparticles, prepared analogously as the Examples 19-24 and A.



Table 5

Component	DMPG	DMPC	Labrafac Hydro	Labrafil 2130CS
	Q	R	S	T

% in Composite	Zeta Potential (mV)	Zeta Potential (mV)	Zeta Potential (mV)	Zeta Potential (mV)
0	-30.3 (Ex. A)	-30.3 (Ex. A)	-30.3 (Ex. A)	-30.3 (Ex. A)
1	-30.0 (Ex. 2)	-30.1	-31.4 (Ex. 13)	
1.5			-33.7	
2			-34.1	
2.5	-30.3		-34.0 (Ex. 14)	
3	-31.7			-21.2 (Ex. 19)
4		-32.2 (Ex. 9)		
4.5	-34 (Ex. 3)			
5	-35.4 (Ex. 4)		-34.2 (Ex. 15)	-13.6
7	-38.2	-31.9 (Ex. 10)		
10	-40.4 (Ex. 5)	-33.7 (Ex. 11)	-33.6 (Ex. 16)	-11.3 (Ex. 21)
12		-34.3		
15				-14.1 (Ex. 22)

Component	DMPG	DMPC	Labrafac Hydro	Labrafil 2130CS
50				-19.8 (Ex. 23)
75				-18.9 (Ex. 24)
100				-25.4

**- Composite nanoparticles morphology according to the invention and according to the prior art.**

In confirmation of the substantial differences between the nanoparticles according to the invention and the prior art, the morphology of some significant samples has been observed (corresponding to the Example 6 and the Example A), by the transmission electronic microscopy technique (TEM), using the "negative coloration" (uracil citrate) process for the preparation of the nanoparticle samples.

In Figure 6 the different morphology of the composite nanoparticles is evident (Example 1, Fig. 6a), with respect to those ones prepared according to the prior art (Example A Fig. 6b): morphologically different, clear and dark areas are in fact noticed on the particles in 6a, corresponding to the phases of the composite material ("preferential segregation"), while the system 6b appears homogeneous.

Further ingredients of the sample corresponding to the Example 1 (Figure 7a and 7b) suggest that the material present on the surface is different from a layer of amphiphilic material adsorbed on the surface of the particle, but it is integral part of the particle itself.

**- Improvement of the oral absorption of peptides (pharmacokinetics and therapeutical efficacy)**

In order to show an important advantage of the invention, composite lipidic nanoparticles containing salmon calcitonin as active principle have been administered "in vivo" (Examples 2, 4, 5), and nanoparticles containing calcitonin prepared according to the prior art (Examples A, B, C).

**DOSAGE PROTOCOL AND SAMPLE ANALYSIS**

Composite nanoparticles suspensions according to the invention (20 ml) containing calcitonin (specific activity: 4000 UI/mg; nominal dose: 600 UI/kg, incorporated effective dose in the nanoparticles: 60-80 UI/kg), have been administered per os to 4 Rhesus macaques. Samples of blood (1.5 ml) were taken at determined times and immediately analyzed after the taking.

Pharmacokinetics: the peptide concentration in the plasma was determined by a specific radioimmunoassay (RIA), expressed as milliunits/ml (mUI/ml).

Therapeutical Efficacy: the levels of the total calcium in the plasma were determined by a colorimetric kit (Cobas Mira, Roche, CH). The levels of ionized calcium were determined by injection in a device equipped with ion-selective membranes (IG Radiometer, Copenhagen, DK). The results were expressed as per cent variation of the calcium levels with respect to the base line.

The results are reported in the Figures 8-11 related to the Examples U-Z1 reported below.

#### EXAMPLE U

Figure 8. Kinetics of absorption of calcitonin formulated in compositions according to the invention (Examples 2, 4, 5) and in compositions according to the prior art (Examples A, B, C), after oral administration.

#### EXAMPLE V

Figure 9. Kinetics of total calcium variation in blood after per os administration of calcitonin formulated in compositions according to the invention (Examples 2, 4, 5) and in compositions according to the prior art (Examples A, B, C).

#### EXAMPLE Z

Figure 10. Kinetics of ionized calcium variation in blood after per os administration of calcitonin formulated in compositions according to the invention (Examples 2, 4, 5) and in compositions according to the prior art (Examples A, B).

#### EXAMPLE Z1

Figure 11. Bioavailability, expressed as AUC (0-8 hours) (area subtended to the plasmatic kinetics curve) related to the oral administration of calcitonin formulated in compositions according to the invention (Examples 2, 4, 5, 7) and in compositions according to the prior art (Examples A, B and C).

It turns out to be evident from the pharmacokinetical reported tests that the bioavailability of the calcitonin formulated in composite nanoparticles according to the invention and administered per os, is significantly increased (to 13.5 times) with respect to the formulations of the prior art. Moreover, it is clear that the nanoparticles according to the invention allow, beside an absorption increment, also a control of the peptide absorption which is present in active form in plasma to 8 hours from the administration. The therapeutical efficacy tests, moreover, point out how the effect on the haematic calcium is greater for the formulations of the invention (Examples 2, 4, 5) with respect to the formulations according to the prior art (Examples A, B, C), and it does not depend on the simple mixture of the components (Example C), but on the composite material presence.

**CLAIMS**

- 1 1. Pharmaceutical compositions in form of solid nanoparticles, characterized in  
2 that they comprise a composite material, consisting of at least one lipidic  
3 substance and at least one amphiphilic substance, and a hydrosoluble, liposoluble  
4 or poorly soluble pharmaceutically active principle.
- 1 2. Compositions as claimed in claim 1, characterized in that said lipidic substance  
2 is present in amounts from 0.5 to 99.5% and that said amphiphilic substance is  
3 present in amounts from 0.5 to 99.5% and said active principle is present in  
4 amounts from 0.001 to 99% by weight with respect to the sum of said lipidic  
5 substance and of said amphiphilic substance. \_
- 1 3. Compositions as claimed in claim 1, characterized in that said nanoparticles  
2 have diameter lower than 1000 nm, and preferably ranging from 50 to 500 nm.
- 1 4. Compositions as claimed in claim 1, comprising: 1) a mixture of two or more  
2 materials of which at least one lipidic material and one amphiphilic material which  
3 form a composite material; 2) one or more surfactants and cosurfactants; 3) a  
4 dispersing/suspending aqueous or non aqueous phase; 4) one or more  
5 pharmaceutically active substances.
- 1 5. Compositions as claimed in claim 4 in form of microemulsions having existence  
2 interval at temperatures lower than the melting temperature of the single  
3 components.
- 1 6. Compositions as claimed in claim 1, characterized in that said lipidic substance  
2 is selected from natural fats, partially or totally hydrogenated vegetal oils, semi-  
3 synthetic and synthetic mono-, di- and tri-glycerides containing saturated and/or  
4 unsaturated fatty acids having aliphatic chain length ranging from C<sub>10</sub> to C<sub>22</sub> and  
5 their polyhydroxyethylated derivatives, liquid waxes selected from isopropyl  
6 myristate, isopropyl caprylate, -caprylate, -laurate, -palmitate, -stearate, esters of  
7 fatty acids selected from ethyloleate, oleyloleate, solid waxes selected from  
8 carnauba wax and bees-wax, aliphatic alcohols selected from cetyl alcohol, stearyl  
9 alcohol, lauryl alcohol, cetylstearyl alcohol and their polyhydroxyethylated  
10 derivatives, aliphatic carboxylic acids (C<sub>10</sub>-C<sub>22</sub>) selected from decanoic acid,  
11 lauric acid, palmitic, stearic, docosanoic acid, oleic, linoleic and their

12 polyhydroxyethylated derivatives.

1 7. Compositions as claimed in claim 1, characterized in that said amphiphilic  
2 substance is selected from phosphatidyl glycerol, phosphatidylcholine,  
3 phosphatidic acid, glyceril monostearate, glyceril monooleate, glyceril  
4 palmitostearate, triglycerides and polyhydroxylated triglycerides, esters of fatty  
5 acids and medium chain fatty acids (C<sub>6</sub>-C<sub>12</sub>), polyethylene glycol, poly-  
6 (propyleneoxide) poly-(ethyleneoxide) copolymers, poloxamer, polyvinyl alcohol,  
7 polyacrylates, poly-(methylvinyl ether) -maleic anhydride copolymers, chitosan,  
8 hyaluronic acid, cellulose, starch, xanthan, scleroglucan, gellan, guar gum, locust  
9 bean gum, alginate, dextran, poly-ε-caprolactone, poly-hydroxybutirate, polylactic  
10 acid, polyglycolic acid and copolymers.

1 8. Compositions as claimed in claim 4, characterized in that said dispersing phase  
2 comprises water, as it is or buffered at different pH and ionic strength, aqueous  
3 solutions of hydrophilic, hydrosoluble or hydrodispersable polymers selected from  
4 polyethylene glycol, polyvinyl pyrrolidone, polyacrylic acids, polymethacrylic acids,  
5 copolymers of polyoxyethylene, polyoxypropylene, dextran, xanthan, scleroglucan,  
6 gum arabic, guar gum, chitosan, cellulose and starch derivatives, sorbitol,  
7 mannitol, xylitol, short chain mono or polyhydroxylic aliphatic alcohols, liquid  
8 polyethylene glycols, polyglycolic glycerides, propylene glycol, tetraglycol, and  
9 ethoxydiglycol.

1 9. Compositions as claimed in claim 4, characterized in that said surfactants are  
2 selected from sorbitan-esters of fatty acids, polyoxyethylene sorbitan esters of  
3 fatty acids, copolymers of polypropyleneoxide-polyethyleneoxide, esters of  
4 polyethyleneglycol -glycerol, esters of polyethylene glycol and acids or long chain  
5 aliphatic alcohols, polyglycerid esters, esters of saccharides and fatty acids,  
6 sodium lauryl sulfate, sodium stearate, sodium oleate, sodium glycocholate,  
7 taurodeoxycholate, taurocholate, ursodeoxycholate, lecithins as they are, partially  
8 hydrogenated and hydrogenated, phospholipids and their semisynthetic or  
9 synthetic derivatives and sorbitan esters of fatty acids.

1 10. Compositions as claimed in claim 4, characterized in that said cosurfactants  
2 are selected from ethanol, 2-propanol, n-butanol, isopropanol, butyric acid, valeric

acid, capronic acid, benzyl alcohol, decanoic acid, lauric acid, caprynyl alcohol, lauryl alcohol, esters or ethers of acids or medium-long chain aliphatic alcohols with mono- or polyhydroxylated alcohols.

11. Compositions as claimed in claim 1, characterized in that said active hydrosoluble principles comprise calcitonin, somatostatin, somatotropin (GH), LH-RH analogues, desmopressin (DDAVP), interferon, molgramostin, epidermic growth factor (EGF), nervous growth factor (NGF), insulin, glucagon, toxins or toxoides, antigenic factors of proteic or polysaccharidic kind, heparin, heparin having low molecular weight and heparinoids.

12. Compositions as claimed in claim 1, characterized in that said active liposoluble principles comprise cyclosporin, leuprolide, taxol and derivatives and etoposide.

13. Compositions as claimed in claim 1, characterized in that said active poorly soluble principles comprise acyclovir and ganciclovir.

14. Process for the preparation of pharmaceutical compositions as defined in claim 1 or in claim 4, characterized by:

a) initial comelting or cosolubilization of at least one lipidic substance with at least one amphiphilic substance at the melting temperature of the substances themselves or at least at the melting temperature of one of the two substances when the latter is soluble or dispersable in the former, obtaining a composite material;

b) addition to said melted composite material of an aqueous solution containing one or more surfactants and cosurfactants, heated to the same temperature of the melted composite material, in order to form a microemulsion, or formation of a microemulsion taking to melting temperature the lipidic and amphiphilic components in presence of water and the surfactants and cosurfactants useful to the formation of the microemulsion itself;

c) dissolution or dispersion of the active principle in the starting comelted material, or addition of the active principle to the microemulsion;

d) dispersion of the microemulsion in water or in aqueous medium at a temperature ranging from +1° to +10 °C, or in non aqueous solvents miscible with

18 water at a temperature ranging from -15 to -30°C, with the achievement of a  
19 nanoparticle suspension;

20 e) optionally the nanoparticles are washed with water or aqueous solutions  
21 through a ultrafiltration or dialysis system in order to remove the surfactants, the  
22 cosurfactants and the free drug present in the suspension, and obtained in form of  
23 dry powder by freeze drying or filtration or spray drying or evaporation of the  
24 solvent.

1 15. Process for the preparation of pharmaceutical compositions as defined in the  
2 claim 1 or in claim 4, characterized by:

3 a) co-melting or co-solubilization of at least one lipidic substance with at least one  
4 amphiphilic substance at the melting temperature of the substances themselves or  
5 at least at the melting temperature of one of the substances, when the latter is  
6 soluble or dispersable in the former, obtaining a composite material;

7 b) dispersion of the composite material in an aqueous solution containing  
8 surfactant substances, stabilizing and/or viscosizing substances, by high pressure  
9 homogenization techniques, maintaining the mixture at the melting temperature of  
10 the composite material or at its "softening" temperature, or at temperatures  
11 maintaining the composite material at the solid state;

12 c) cooling of the mixture by passage in a refrigerant circuit at temperatures  
13 ranging from +10 °C to -30 °C obtaining the nanoparticles;

14 d) optionally the nanoparticles are washed with water or aqueous solutions  
15 through an ultrafiltration or dialysis system, which allows to remove the  
16 surfactants, the cosurfactants and the free drug present in the suspension and the  
17 composite nanoparticles are recovered as solid by freeze drying techniques or  
18 filtration techniques or by spray drying techniques or by solvent evaporation  
19 techniques, the active principle being added during the comelting or  
20 cosolubilization step or in the subsequent steps of the process, as it is or in  
21 mixture with substances having surface action which help the incorporation in the  
22 nanoparticles or the adsorption on their surface.

1 16. Compositions as claimed in claim 1, characterized in that said nanoparticles  
2 are dispersed in aqueous suspension at a concentration ranging from 0.1 to 50%



3 w/v.

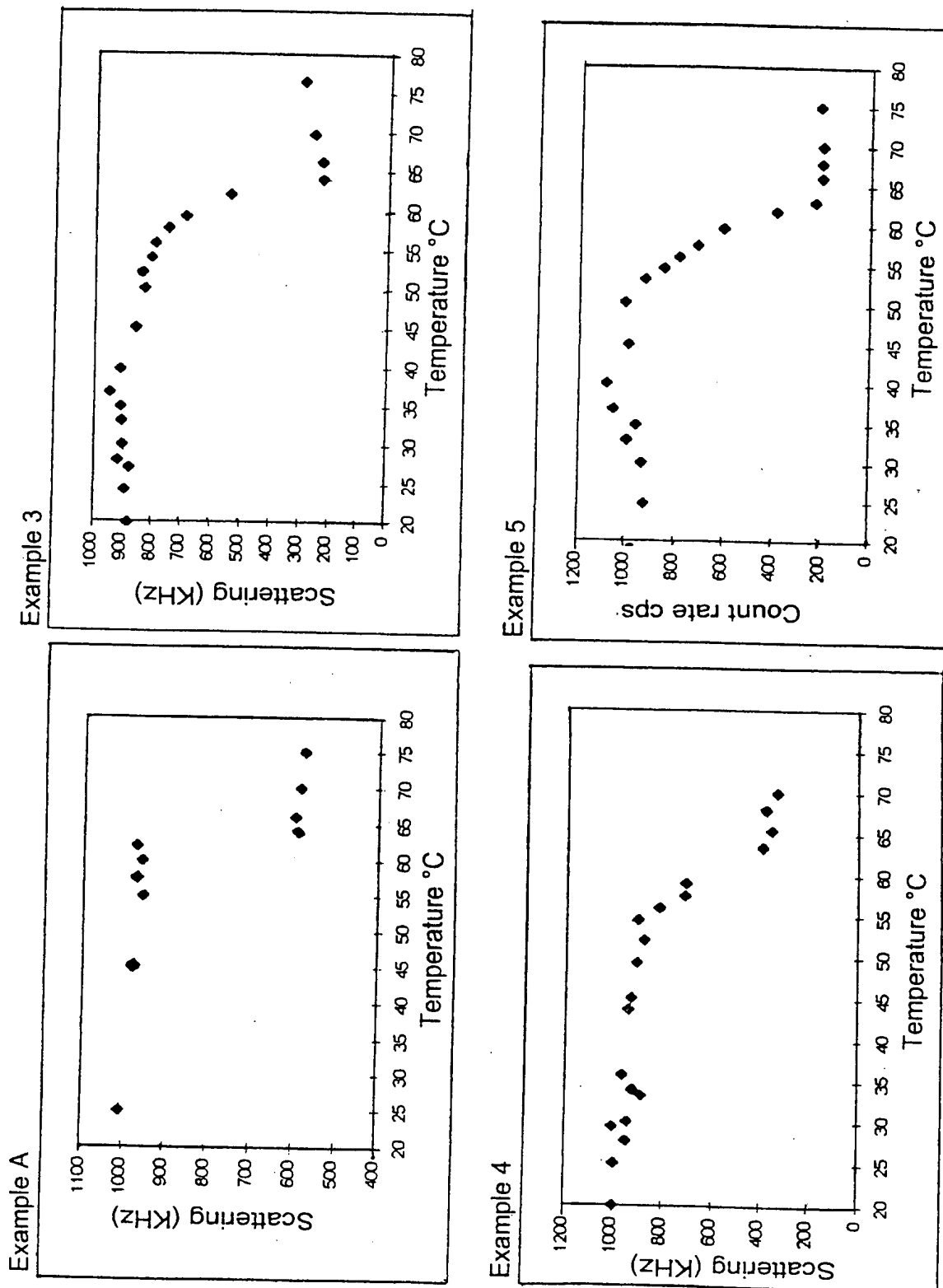
1 17. Compositions as claimed in claim 1, characterized in that said nanoparticles  
2 are formulated as an aqueous suspension inside the capsules or globula for  
3 pharmaceutical use.

1 18. Compositions as claimed in claim 1, characterized in that said nanoparticles  
2 are formulated in syrup form.

1 19. Compositions as claimed in claim 1, characterized in that said nanoparticles  
2 are formulated as solid powder, inside the capsules for pharmaceutical use.

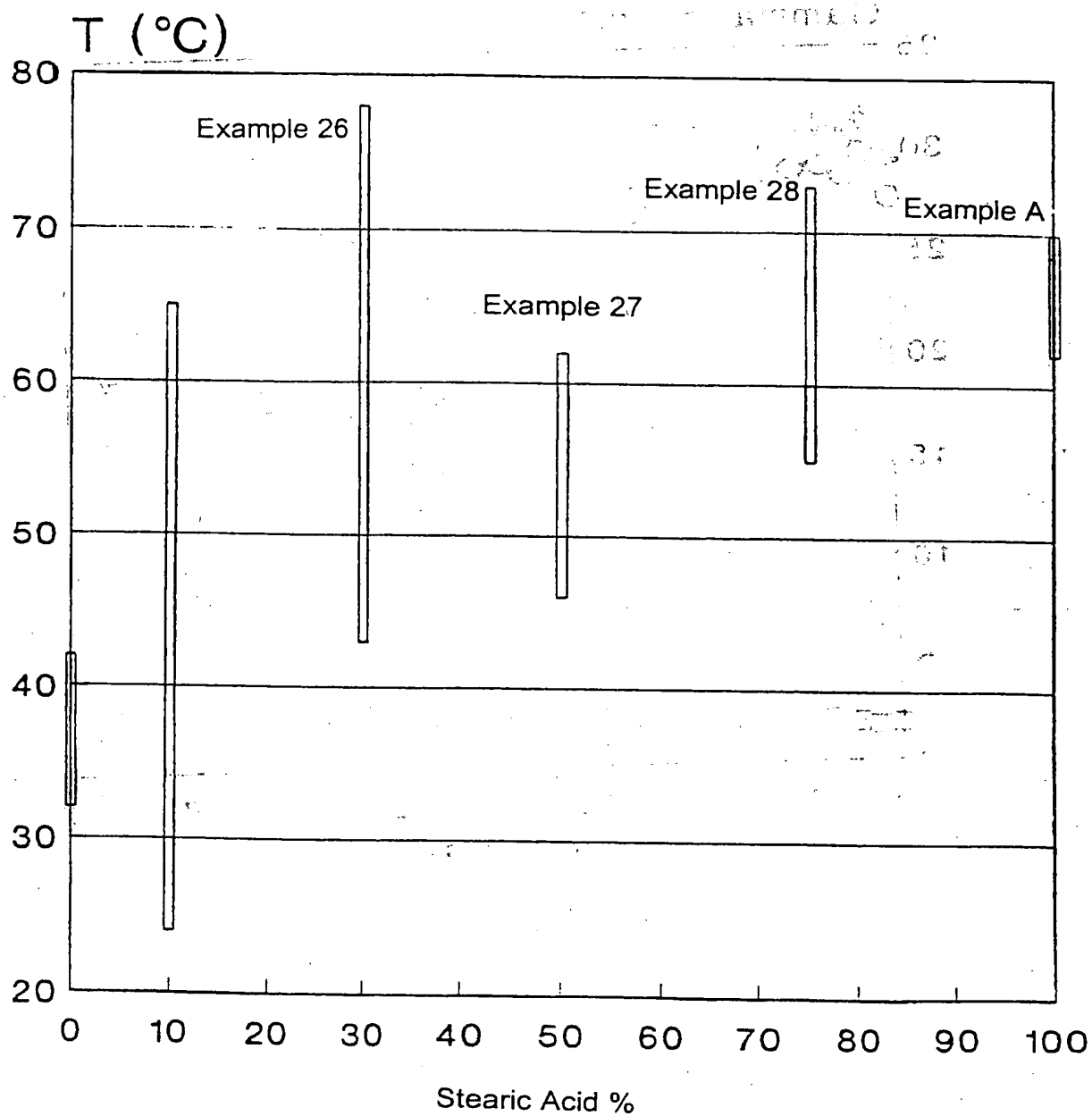
1 20. Compositions as claimed in claim 1, characterized in that said nanoparticles  
2 are formulated as solid powder, inside tablets, pellets or granules for  
3 pharmaceutical use.

Figure 1



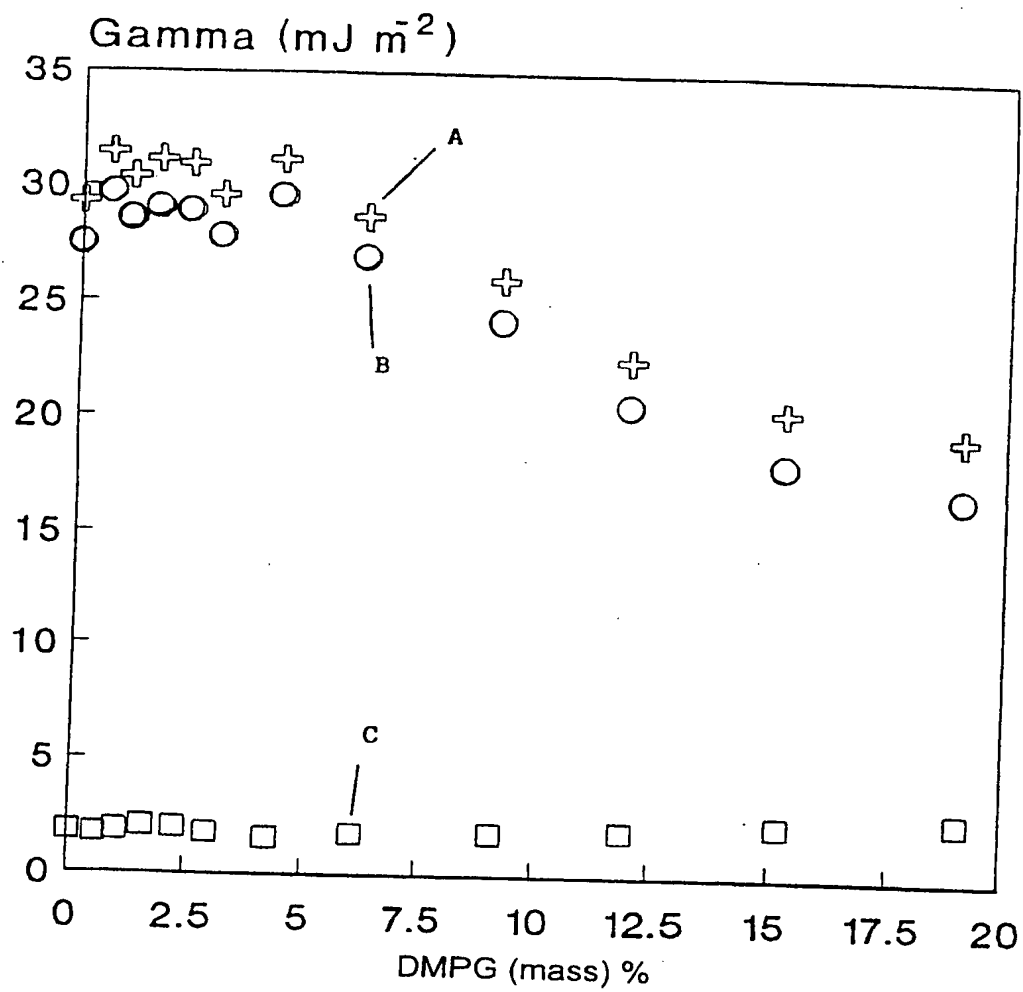
2/11

Figure 2



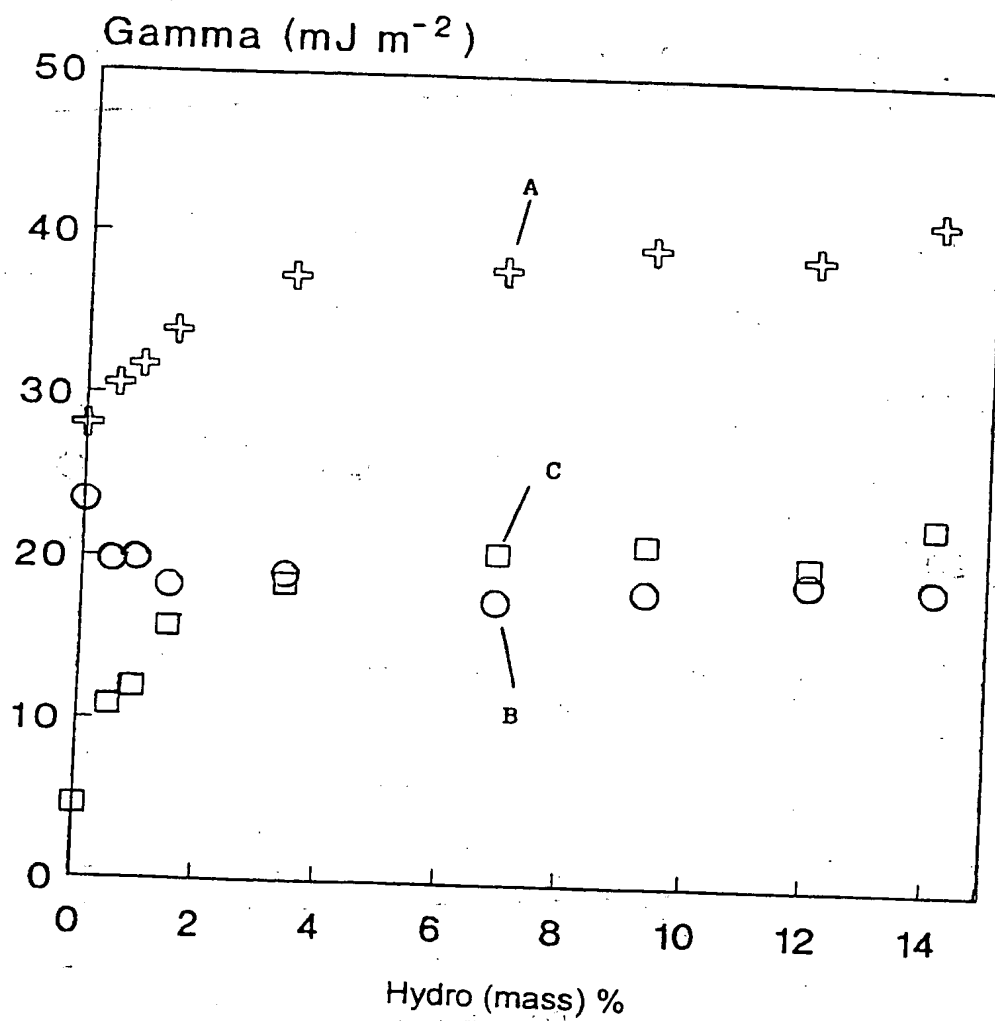
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Figure 3



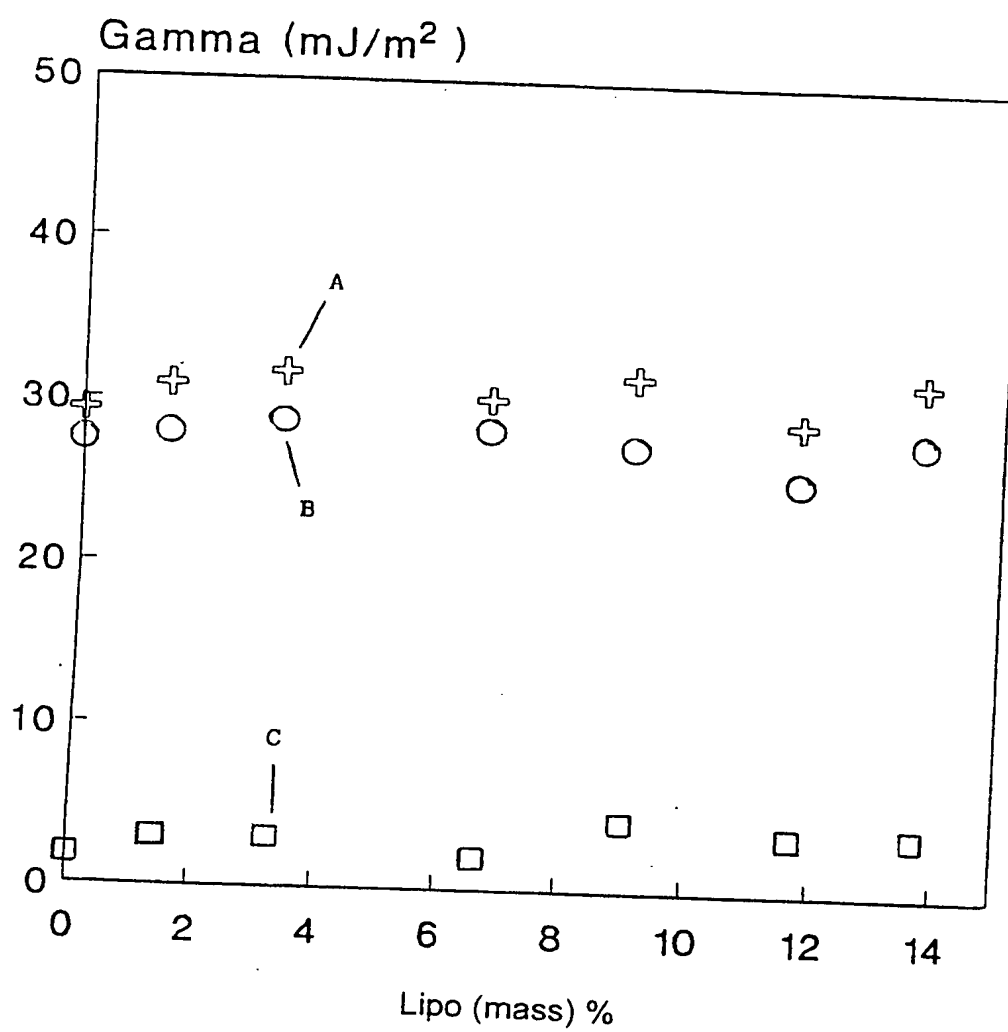
4/11

Figure 4

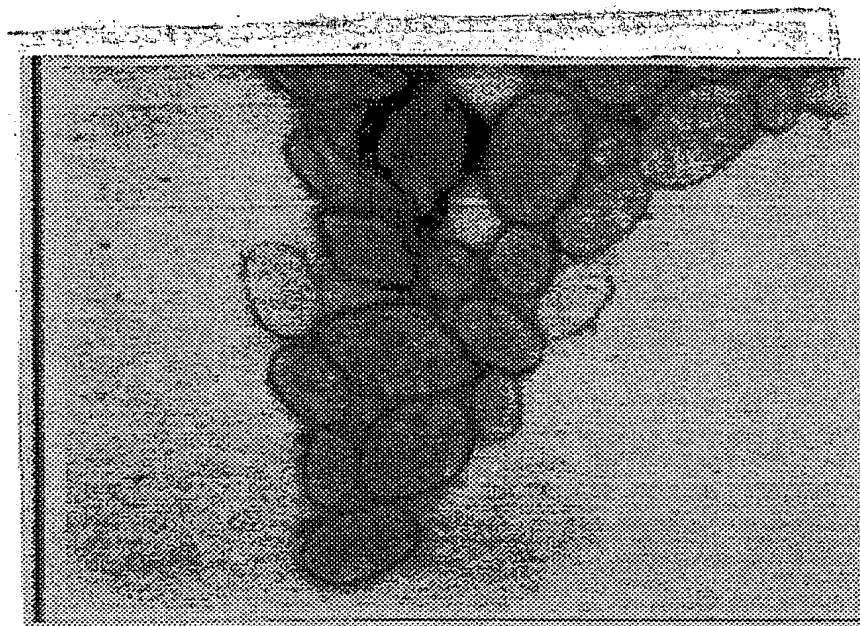


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Figure 5



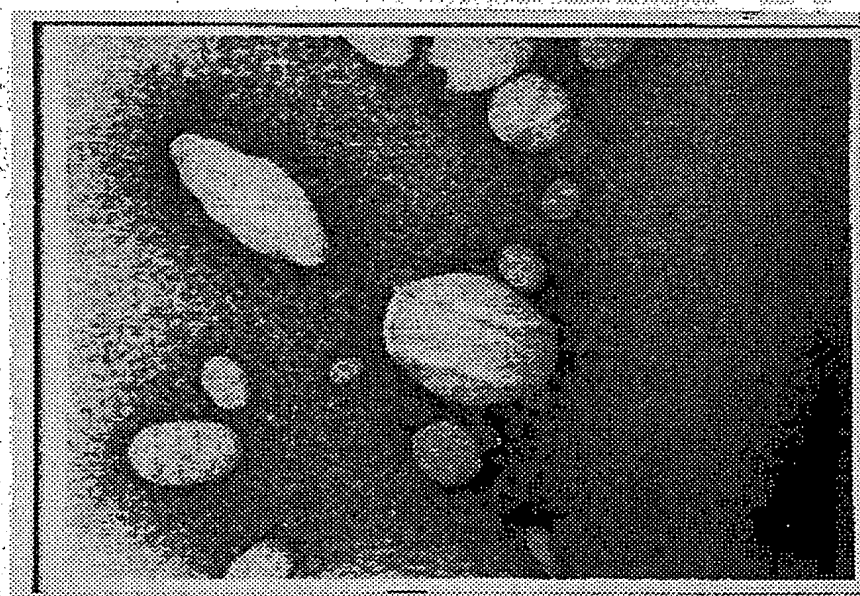
6/11



6a

SA-100X  
8,9 Kx

1µm  
x2



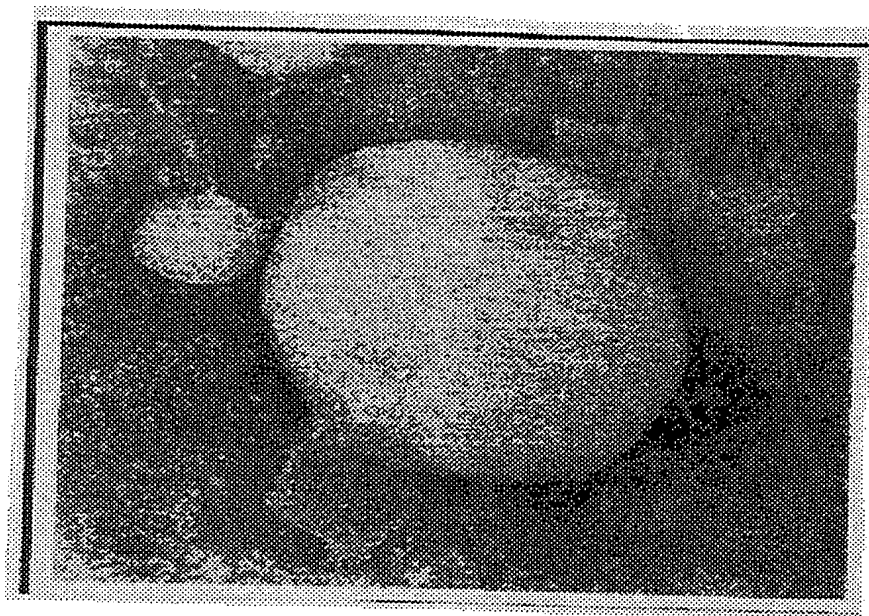
6b

SA-PG10  
11Kx

1µm

Figure 6

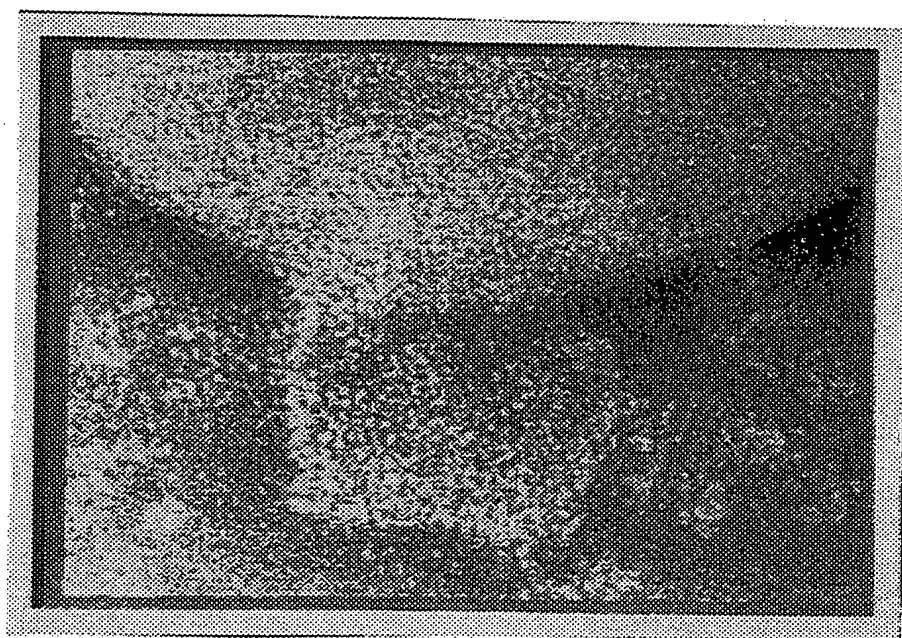
7/11



7a

SA-PG10  
36 Kx

200 nm



7b

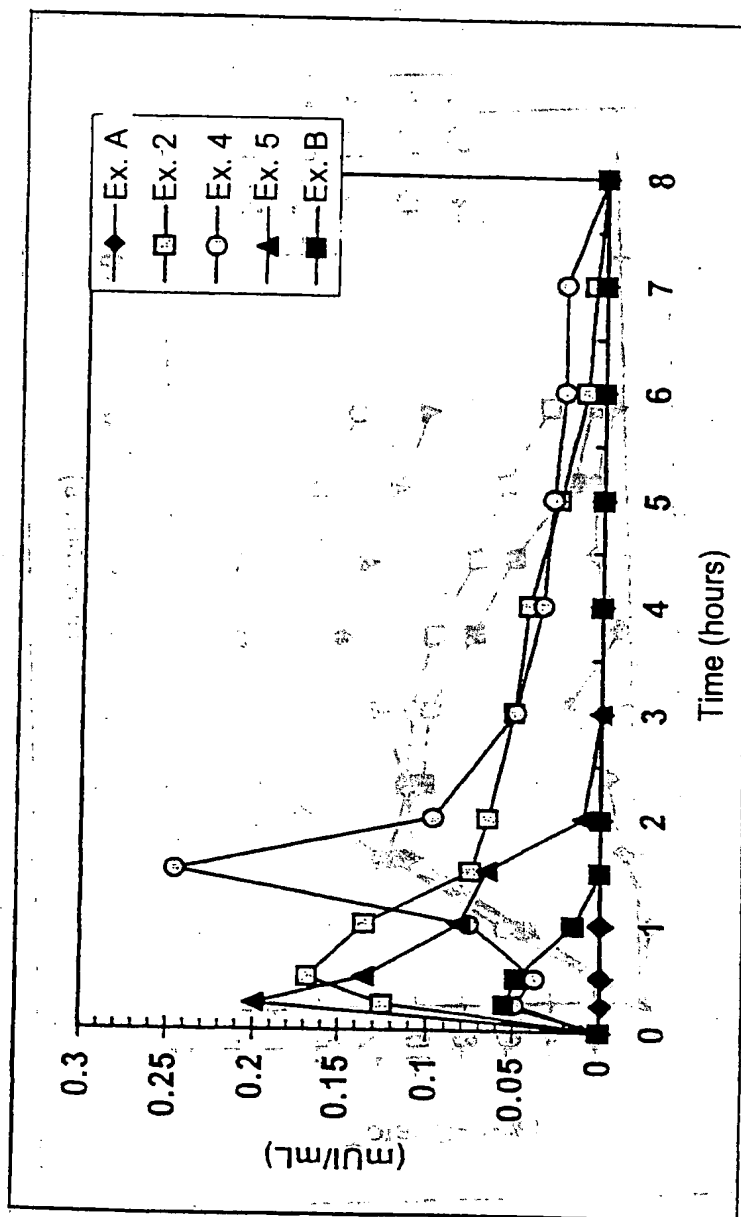
SA-PG10  
89Kx

100nm

Figure 7

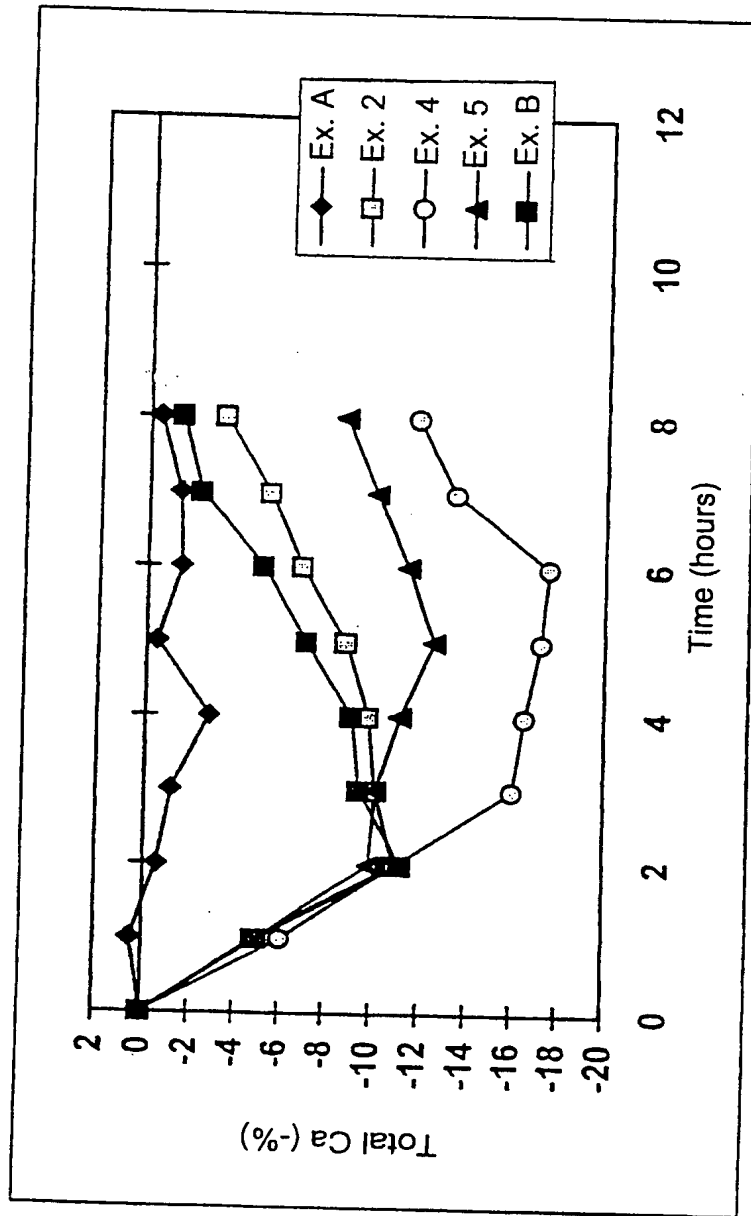


Figure 8



9/11

Figure 9



10/11

Figure 10

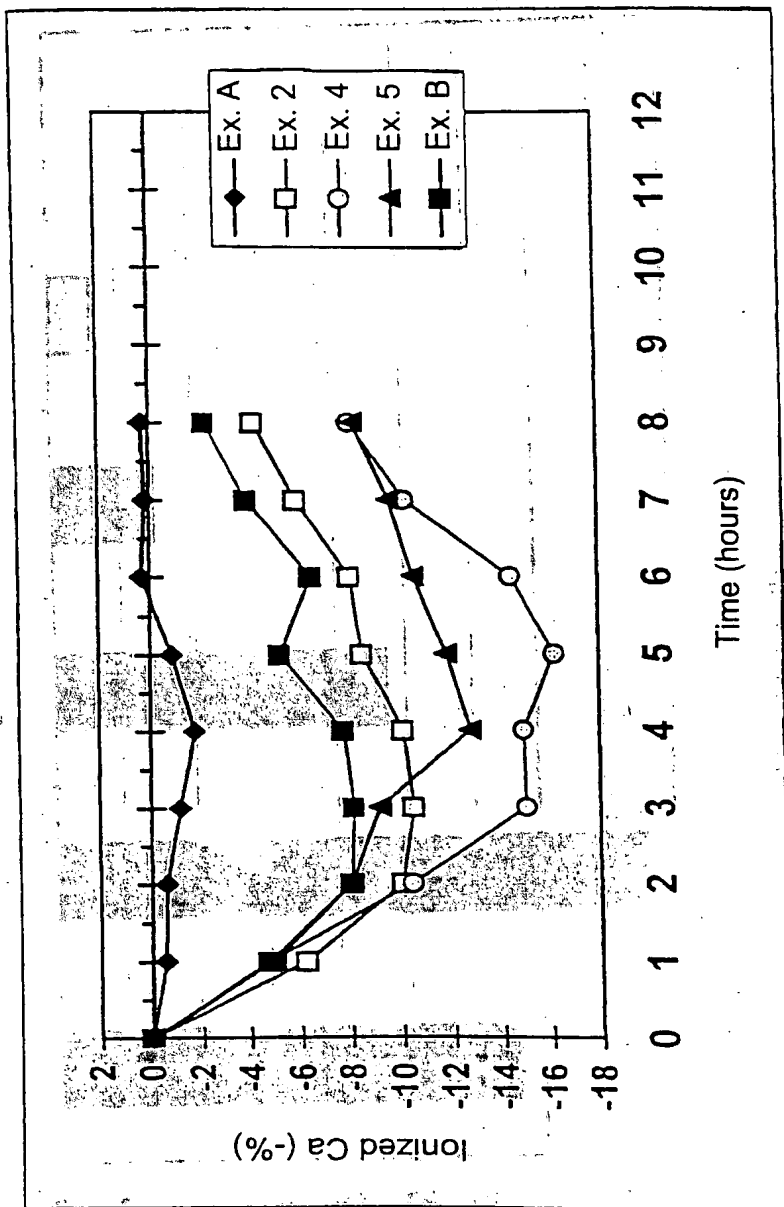
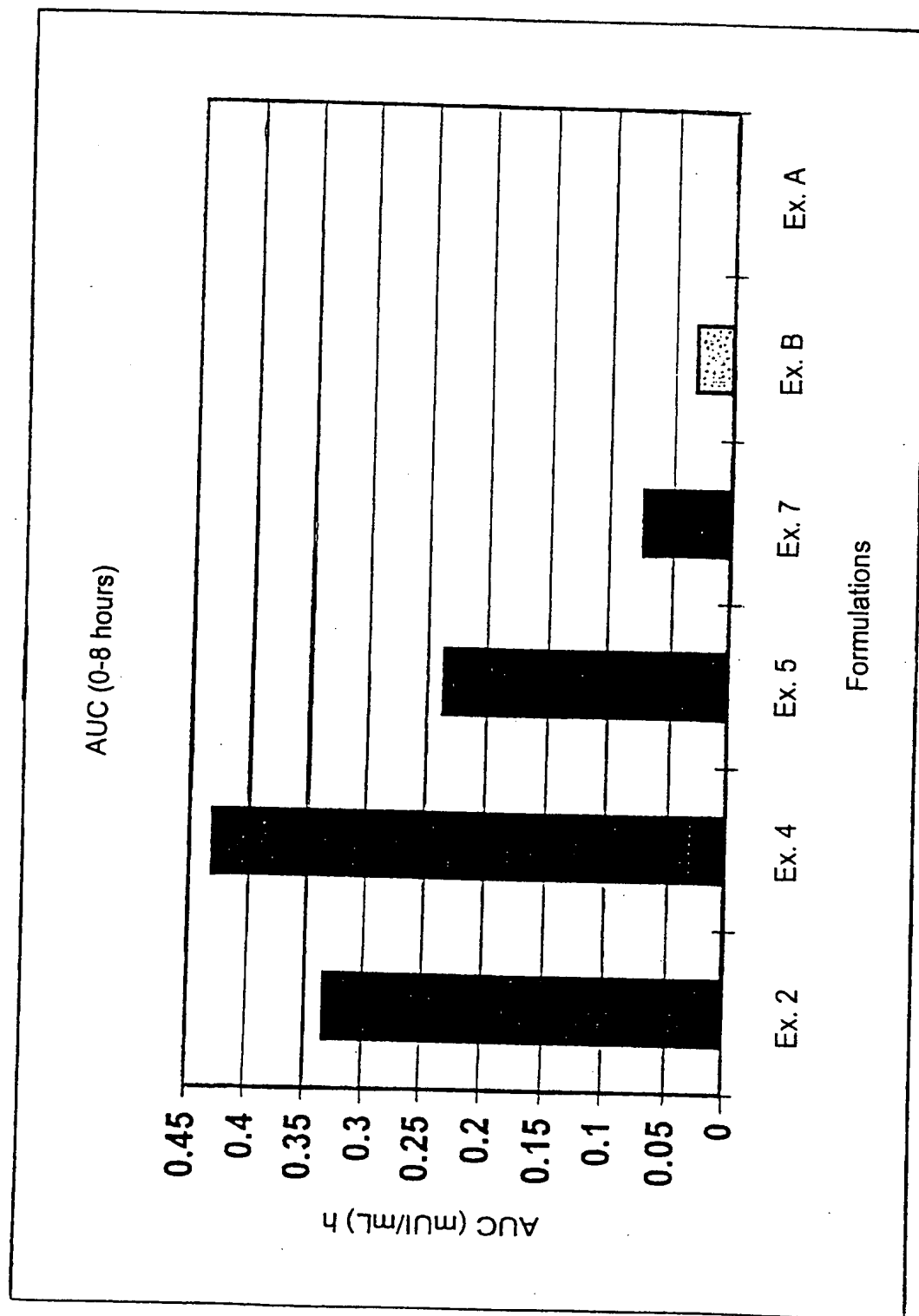


Figure 11



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 99/00782

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 A61K9/51 A61K38/23 A61K31/725 A61K31/12

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 94 20072 A (PHARMACIA AB ; WESTESEN KIRSTEN (DE); SIEKMANN BRITTA (DE)) 15 September 1994 (1994-09-15)	1-4, 6-9, 12, 13, 19
Y	page 1, line 4-10 page 15, line 19, 20 page 15, line 28 page 16, line 8-32 page 18, line 10-13 page 23, line 22-30 examples claims	14-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

16 July 1999

Date of mailing of the international search report

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# INTERNATIONAL SEARCH REPORT

International Application No.

PCT/EP 99/00782

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 526 666 A (GASCO MARIA ROSA) 10 February 1993 (1993-02-10) cited in the application	1-3,6,11
Y	page 2, column 1, line 55 - column 2, line 24 page 2, column 2, line 32-57 page 3, column 3, line 21-36 examples claims	14-16
X	----- BOLTRI L ET AL: "RELEVANT FACTORS AFFECTING THE FORMATION AND GROWTH OF LIPID NANOSPHERES SUSPENSIONS" EUROPEAN JOURNAL OF PHARMACEUTICS AND BIOPHARMACEUTICS, vol. 41, no. 1, 1 January 1995 (1995-01-01), pages 70-75, XP000482895 ISSN: 0939-6411 abstract page 70, "introduction" page 70-71, paragraph 2.2 "Preparation of lipid nanospheres and their storage"	1,3,6
X	--- US 4 880 634 A (SPEISER PETER) 14 November 1989 (1989-11-14) column 2, line 58 - column 3, line 28 column 6, line 50 column 8, line 4-47 examples claims -----	1-3,6,7, 13

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 99/00782

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9420072	A	15-09-1994	CA 2091152 A	06-09-1994
			AU 676279 B	06-03-1997
			AU 6225394 A	26-09-1994
			EP 0687172 A	20-12-1995
			FI 954143 A	19-10-1995
			JP 8507515 T	13-08-1996
			NO 953461 A	06-11-1995
			NZ 262541 A	24-04-1997
			US 5785976 A	28-07-1994
			US 5885486 A	23-03-1999
EP 0526666	A	10-02-1993	AT 136774 T	15-05-1996
			DE 69118880 D	23-05-1996
			DE 69118880 T	07-11-1996
			ES 2089066 T	01-10-1996
			US 5250236 A	05-10-1993
US 4880634	A	14-11-1989	DE 3421468 A	19-12-1985
			AT 55243 T	15-08-1990
			EP 0167825 A	15-01-1986
			JP 61056122 A	20-03-1986